414 Rec'd PCT/PTO 20 SEP 2000

Form PTO-1390 (REV 12-29-99) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 0623.0890000/EKS/PSC
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO (IF KNOWN, SEE 37 C F.R § 1 5) To be assigned 09/646679
INTERNATIONAL APPLICATION NO PCT/GB99/00905	INTERNATIONAL FILING DATE 22 March 1999	PRIORITY DATE CLAIMED 20 March 1998

TITLE OF INVENTION
Signal Transduction Protein Involved in Plant Dehiscence

APPLICANT(S) FOR DO/EO/US
Wyatt, Paul; Roberts, Jeremy Alan; and Whitelaw, Catherine

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 372(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ A copy of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment and submission of sequence listing.
    ☒ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: Authorization To Treat A Reply As Incorporating An Extension Of Time Under 37 C.F.R. § 1.136(a)(3) *(in duplicate)*; and Claim for Priority Under 35 U.S.C. § 119(a)-(d) In Utility Application, with Certified Priority Document attached.

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U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.50) To be assigned 09/646679	INTERNATIONAL APPLICATION NO PCT/GB99/00905	ATTORNEY'S DOCKET NUMBER 0623.0890000/EKS/PSC

17. ☒ The following fees are submitted:				CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .... **$970.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .... **$840.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... **$690.00** International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) .... **$670.00** International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .... **$ 96.00**					
ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$ 130.00	
Claims	Number Filed	Number Extra	Rate		
Total Claims	29 - 20 =	9	X **$18.00**	$ 162.00	
Independent Claims	3 - 3 =	0	X **$78.00**	$ 0.00	
Multiple dependent claim(s) (if applicable)			+ **$260.00**	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1,132.00	
Reduction of ½ for filing by small entity, if applicable. A Small Entity Statement must be filed. (Note 37 CFR 1.9, 1.27, 1.28)				$	
SUBTOTAL =				$ 1,132.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 1,132.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 1,132.00	
				Amount to be: refunded:	$
				charged:	$

a. ☒ A check in the amount of $1,132.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. ________ in the amount of $ ________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0036. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit Under 37 CFR 1.494 or 1.495 has not been met, a *petition to revive* (37 CFR 1.137(a) or (b)) *must be filed and granted to restore the application to pending status.*

SEND ALL CORRESPONDENCE TO:
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW, Suite 600
Washington, D.C. 20005-3934

Eric K. Steffe 9/20/00
SIGNATURE

Eric K. Steffe
NAME

36,688
REGISTRATION NUMBER

09/646679

534 Rec'd PCT/PTO 20 SEP 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Wyatt *et al.*	Art Unit: To be assigned
Appl. No. To be assigned (U.S. National Phase of PCT/GB99/00905)	Examiner: To be assigned
Filed: Herewith	Atty. Docket: 0623.0890000/EKS/PSC
For: **Signal Transduction Protein Involved in Plant Dehiscence**	

Second Preliminary Amendment

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution please amend the application as follows:

In the Claims

Please amend the claims as follows:

3. Nucleic acid as claimed in claim 1 [or claim 2] which is naturally expressed in a dehiscence zone.

4. Nucleic acid <u>as claimed in claim 1</u> encoding a protein wherein the protein:

a) comprises the amino acid sequence shown in Figure 1 or;

b) has one or more amino acid deletions, insertions or substitutions relative to a protein as defined in a) above, and has at least 40% amino acid sequence identity therewith; or

c) a fragment of a protein as defined in a) or b) above which is at least 10 amino acids long.

5. Nucleic acid as claimed in [any one of claims 1 to 4] claim 1 which comprises the sequence set out in Figure 1 or a fragment thereof which is at least 30 bases long.

6. Nucleic acid, as claimed in [any one of claims 1 to 5] claim 1 in combination with one or more further nucleic acid sequence which is dehiscence-zone expressed.

7. Nucleic acid which is antisense to nucleic acid as claimed in [any one of claims 1 to 6] claim 1.

8. Nucleic acid as claimed in [any one of claims 1 to 7] claim 1 including a promoter or other regulatory sequence which controls expression of the nucleic acid.

9. Nucleic acid which is the naturally occurring promoter or other regulatory sequence which controls expression of nucleic acid as claimed in [any one claims 1 to 8] claim 1.

10. Nucleic acid as claimed in [any one of claims 1 to 9] claim 1 which is in the form of a vector.

11. A cell comprising nucleic acid as claimed in [any one of claims 1 to 10] claim 1.

13. A process for obtaining a cell [as claimed in claim 11 or claim 12] comprising introducing nucleic acid as claimed in [any one of claims 1 to 10] claim 1 into said cell.

14. A plant or a part thereof comprising a cell as claimed in [claim 11 or] claim 12.

15. Propagating material or a seed comprising a cell as claimed in [claim 11 or] claim 12.

16. A process for obtaining a plant or plant part [as claimed in claim 14 or claim 15] comprising obtaining a cell as claimed in claim 11 and growth thereof [or obtaining a plant, plant part, or propagating material as claimed in claim 14 or claim 15 and growth thereof].

18. A protein as claimed in claim 17 which:

a) comprises the amino acid sequence shown in Figure 1 or;

b) has one or more amino acid deletions, insertions or substitutions relative to a protein as defined in a) above, and has at least 40% amino acid sequence identity therewith; or

c) a fragment of a protein as defined in a) or b) above which is at least 10 amino acids long.

19. A plant as claimed in claim 17 [or claim 18] which is isolated or recombinant.

21. A process [as claimed in claim 20] for regulating or controlling dehiscence in a plant or plant part which comprises obtaining a plant cell as claimed in [claim 21 or part of a plant as claimed in claim 14] claim 12 and deriving a plant therefrom.

22. A process for regulating or controlling dehiscence in a plant or plant part [as claimed in claim 20] which comprises obtaining propagating material or a seed as claimed in claim 15 and deriving a plant therefrom.

24. A process for controlling or regulating plant dehiscence comprising introducing the [Use of] nucleic acid as claimed in [any one of claims 1 to 10] claim 1 [in the control/regulation of plant dehiscence] into a cell, tissue, plant part thereof or propagating material and expressing said nucleic acid.

25. [Use of nucleic] Nucleic acid as claimed in [any one of claims 1 to 10 as] claim 4 which is a probe.

26. A process for producing a cell, tissue, plant part thereof or propagating material comprising introducing the [Use of] nucleic acid as claimed in [any one claims 1 to 10] claim 1 into a cell, tissue, plant part thereof or propagating material and causing growth of said cell, tissue, plant part thereof or propagating material [in the production of a cell, tissue, plant part thereof or propagating material].

29. [Use of a] A protein as claimed in [any one of claims 17 to 19] claim 18 which is [as] a probe.

Remarks

Claims 3-11, 13-16, 18-19, 21-22, 24-26, and 29 have been amended to delete multiple dependencies . The amendments do not add new matter and Applicants respectfully request that they be entered. Support for the amendments can be found throughout the specification (e.g. in the original claims). Upon entry of the forgoing amendment, claims 1-29 are pending in the application with claims 1, 17 and 27 being the independent claims.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor are hereby authorized to be charged to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Eric K. Steffe
Attorney for Applicants
Registration No. 36,688

Date: 9/20/00

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09/646679

534 Rec'd PCT/PTO 20 SEP 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Wyatt *et al.*

Appl. No. To be assigned (U.S. National Phase of PCT/GB99/00905)

Filed: Herewith

For: **Signal Transduction Protein Involved in Plant Dehiscence**

Art Unit: To be assigned

Examiner: To be assigned

Atty. Docket: 0623.0890000/EKS/PSC

Claim For Priority Under 35 U.S.C. § 119(a)-(d) In Utility Application

Commissioner for Patents
Washington, D.C. 20231

Sir:

Priority under 35 U.S.C. § 119(a)-(d) is hereby claimed to the following priority document(s), filed in a foreign country within twelve (12) months prior to the filing of the above-referenced United States utility patent application:

Country	Priority Document Appl. No.	Filing Date
Great Britain	GB9806113.8	20 March 1998

A certified copy of each listed priority document is submitted herewith. Prompt acknowledgment of this claim and submission is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, ~~GOLDSTEIN~~ & FOX P.L.L.C.

Eric K. Steffe
Attorney for Applicants
Registration No. 36,688

Date: 9/20/00

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(202) 371-2600

09/646679

534 Rec'd PCT/PTO 20 SEP 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Wyatt *et al.*	Art Unit: To be assigned
Appl. No. To be assigned (U.S. Natl. Phase of PCT/GB99/00905)	Examiner: To be assigned
Filed: Herewith	Atty. Docket: 0623.0890000/EKS/PSC
For: **Signal Protein Involved in Plant Dehiscence**	

Preliminary Amendment and Submission of Sequence Listing

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution, please amend the application as follows:

In the Specification:

Please insert the sequence listing at the end of the application.

On page 4, line 24, after "figure 1 ", please insert --(SEQ ID NO: 15)--.

On page 5, line 10, after "Figure 1 ", please insert --(SEQ ID NO: 15)--.

On page 6, line 25, after "figure 1 ", please insert --(SEQ ID NO: 15)--.

On page 8, line 17, after "figure 1 ", please insert --(SEQ ID NO: 14)--.

On page 8, line 19, after "Figure 1 ", please insert --(SEQ ID NO: 14)--.

On page 9, line 22, after "figure 1 ", please insert --(SEQ ID NO: 14)--.

On page 12, line 10, after "figure 1 ", please insert --(SEQ ID NO: 15)--.

On page 12, line 22, after "Figure 1 ", please insert --(SEQ ID NO: 15)--.

On page 14, line 12, after "Fig 5", please insert -- (SEQ ID NOs: 3, 5, and 6)--.

On page 14, line 12, after "Fig 9", please insert -- (SEQ ID NOs: 37 and 38)--.

On page 14, line 12, after "Fig 11", please insert -- (SEQ ID NOs: 7 and 8)--.

On page 18, line 15, after "Figure 15)", please insert --(SEQ ID NOs: 31 and 32)--.

On page 18, line 16, after "16)", please insert --(SEQ ID NOs: 33 and 34)--.

On page 18, line 16, after "(Figure 17)", please insert --(SEQ ID NOs: 35 and 36)--.

On page 19, line 9, after "Sac66", please insert --(SEQ ID NO: 31)--.

On page 19, line 9, after "DZ15", please insert --(SEQ ID NO: 33)--.

On page 19, line 10, after "OSR(7)", please insert --(SEQ ID NO: 35)--.

On page 19, line 22, after "sites", please insert --(SEQ ID NOs: 14 and 15)--.

On page 20, line 7, after "DZ2B", please insert --(SEQ ID NO: 23)--.

On page 20, line 16, after "DZ2AT3 peptide", please insert --(SEQ ID NOs: 27 and 28)--.

On page 20, line 20, after "DZ2AT3", please insert --(SEQ ID NO: 29)--.

On page 21, line 2, after "Sac66", please insert --(SEQ ID NOs: 31 and 32)--.

On page 21, line 4, after "DZ15", please insert --(SEQ ID NOs: 33 and 34)--.

On page 21, line 6, after "OSR7", please insert --(SEQ ID NOs: 35 and 36)--.

On page 22, line 22, after "TGG-3')", please insert --(SEQ ID NO: 1)--.

On page 23, line 1, after "TGG-3')", please insert --(SEQ ID NO: 1)--.

On page 23, line 1, after "CGA A -3'), please insert --(SEQ ID NO: 2)--.

On page 23, line 14, after "TGG-3')", please insert --(SEQ ID NO: 1)--.

On page 23, line 14, after "CGA A -3')", please insert --(SEQ ID NO: 2)--.

On page 23, line 18, after "(Figure 1)", please insert -- (SEQ ID NO: 14)--.

On page 24, line 3, after "(Figure1)", please insert -- (SEQ ID NO: 14)--.

On page 25, line 15, after the first "(Figure 5)", please insert --(SEQ ID NO: 23)--.

On page 25, line 18, after "DZ2BFL", please insert -- (SEQ ID NO: 3)--.

On page 25, line 19, after "T7", please insert -- (SEQ ID NO: 4)--.

On page 26, line 13, after "DZ2BGENF", please insert -- (SEQ ID NO: 5)--.

On page 26, line 15, after "DZ2BGENR", please insert -- (SEQ ID NO: 6)--.

On page 27, line 7, after "Figure 9", please insert -- (SEQ ID NO: 27)--.

On page 27, line 28, after "(Figure 11)", please insert -- (SEQ ID NO: 29)--.

On page 28, line 1, after "ATDZ2F", please insert -- (SEQ ID NO: 7)--.

On page 28, line 2, after "ATDZ2R", please insert -- (SEQ ID NO: 8)--.

On page 30, line 5, after "DZ2FLA", please insert -- (SEQ ID NO: 9)--.

On page 30, line 6, after "DZ2RLA", please insert -- (SEQ ID NO: 10)--.

On page 31, line 15, after "Figure 15)", please insert -- (SEQ ID NOs: 31 and 32)--.

On page 31, line 16, after "16)", please insert -- (SEQ ID NOs: 33 and 34)--.

On page 31, line 16, after "Figure 17", please insert -- (SEQ ID NOs: 35 and 36)--.

On page 32, line 8, after "Figure 16)", please insert -- (SEQ ID NO: 33)--.

On page 32, line 15, after "T7", please insert -- (SEQ ID NO: 4)--.

On page 32, line 16, after "DZ15RL", please insert -- (SEQ ID NO: 11)--.

On page 32, line 25, after "F1", please insert -- (SEQ ID NO: 12)--.

On page 32, line 26, after "RI", please insert -- (SEQ ID NO: 13)--.

Remarks

The specification has been amended to direct the entry of this sequence listing after the claims of the above identified application and to provide sequence identifiers in all instances where the specification discusses the sequences. *See* 37 C.F.R. § 1.821(d).

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter. In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same.

It is respectfully believed that this application is now in condition for examination. Early notice to this effect is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Eric K. Steffe

Eric K. Steffe
Attorney for Applicants
Registration No. 36,688

Date: 9/20/00

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SKGF Rev. 12/30/99 mac

09/646679

SEQUENCE LISTING

<110> Wyatt, Paul
Roberts, Jeremy A.
Whitelaw, Catherine

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Ser Ile Cys Arg Arg Leu Arg Ser Gln Ser Asn Pro Met Pro Ile Ile
65   70   75   80

Met Val Thr Ala Lys Gly Glu Glu Val Asp Arg Ile Val Gly Leu Glu
                85   90   95

Ile Gly Ala Asp Asp Tyr Ile Pro Lys Pro Phe Asn Pro Arg Glu Leu
            100   105   110

Leu Ala Arg Ile Arg Ala Val Leu Arg Arg Gln Ala Asn Glu Leu Pro
115 120 125

Gly Ala Pro Ser
130

<210> 18
<211> 126
<212> PRT
<213> Escherichia coli

<400> 18
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1 5 10 15

Met Val Cys Phe Val Leu Glu Gln Asn Gly Phe Gln Pro Val Glu Ala
20 25 30

Glu Asp Tyr Asp Ser Ala Val Asn Gln Leu Asn Glu Pro Trp Pro Asp
35 40 45

Leu Ile Leu Leu Asp Trp Met Leu Pro Gly Gly Ser Gly Ile Gln Phe
50 55 60

Ile Lys His Leu Lys Arg Glu Ser Met Thr Arg Asp Ile Pro Val Val
65 70 75 80

Met Leu Thr Ala Arg Gly Glu Glu Glu Asp Arg Val Arg Gly Leu Glu
85 90 95

Thr Gly Ala Asp Asp Tyr Ile Thr Lys Pro Phe Ser Pro Lys Glu Leu
100 105 110

Val Ala Arg Ile Lys Ala Val Met Arg Arg Ile Ser Pro Met
115 120 125

<210> 19
<211> 144
<212> PRT
<213> Salmonella typhimurium

<400> 19
Met Gln Arg Gly Ile Val Trp Val Val Asp Asp Asp Ser Ser Ile Arg
1 5 10 15

Trp Val Leu Glu Arg Ala Leu Ala Gly Ala Gly Leu Thr Cys Thr Thr
20 25 30

Phe Glu Asn Gly Asn Asn Thr Arg Cys Glu Val Leu Ala Ala Leu Ala
35 40 45

Ser Lys Thr Pro Asp Val Leu Leu Ser Asp Ile Arg Met Pro Gly Met
50 55 60

Asp Gly Leu Ala Leu Leu Lys Gln Ile Lys Gln Arg His Pro Met Leu
65 70 75 80

Pro Val Ile Ile Met Thr Ala Asn Thr Arg Cys His Ser Asp Leu Asp
85 90 95

Ala Ala Val Ser Ala Tyr Gln Gln Gly Ala Phe Asp Tyr Leu Pro Lys
100 105 110

Pro Phe Asp Ile Asp Glu Ala Val Ala Leu Val Glu Arg Ala Ile Ser
115 120 125

His Tyr Gln Glu Gln Gln Gln Pro Arg Asn Ile Glu Val Asn Gly Pro
130 135 140

<210> 20
<211> 124
<212> PRT
<213> Bacillus subtilis

<400> 20
Met Met Asn Glu Lys Ile Leu Ile Val Asp Asp Gln Tyr Gly Ile Arg
1 5 10 15

Ile Leu Leu Asn Glu Val Phe Asn Lys Glu Gly Tyr Gln Thr Phe Gln
20 25 30

Ala Ala Asn Gly Leu Gln Ala Leu Asp Ile Val Thr Lys Glu Arg Pro
35 40 45

Asp Leu Val Leu Leu Asp Met Lys Ile Pro Gly Met Asp Gly Ile Glu
50 55 60

Ile Leu Lys Arg Met Lys Val Ile Asp Glu Asn Ile Arg Val Ile Ile
65 70 75 80

Met Thr Ala Tyr Gly Glu Leu Asp Met Ile Gln Glu Ser Lys Glu Leu
85 90 95

Gly Ala Leu Thr His Phe Ala Lys Pro Phe Asp Ile Asp Glu Ile Arg
100 105 110

Asp Ala Val Lys Lys Tyr Leu Pro Leu Lys Ser Asn
115 120

<210> 21
<211> 129
<212> PRT
<213> Escherichia coli

<400> 21
Met Ala Asp Lys Glu Leu Lys Phe Leu Val Val Asp Asp Phe Ser Thr

1 5 10 15

Met Arg Arg Ile Val Arg Asn Leu Leu Lys Glu Leu Gly Phe Asn Asn
20 25 30

Val Glu Glu Ala Glu Asp Gly Val Asp Ala Leu Asn Lys Leu Gln Ala
35 40 45

Gly Gly Tyr Gly Phe Val Ile Ser Asp Trp Asn Met Pro Asn Met Asp
50 55 60

Gly Leu Glu Leu Leu Lys Thr Ile Arg Ala Asp Gly Ala Met Ser Ala
65 70 75 80

Leu Pro Val Leu Met Val Thr Ala Glu Ala Lys Lys Glu Asn Ile Ile
85 90 95

Ala Ala Ala Gln Ala Gly Ala Ser Gly Tyr Val Val Lys Pro Phe Thr
100 105 110

Pro Ala Thr Leu Glu Glu Lys Leu Asn Lys Ile Phe Glu Lys Leu Gly
115 120 125

Met

<210> 22
<211> 111
<212> PRT
<213> Arabidopsis thaliana

<220>
<221> Unsure
<222> 67
<223> Xaa= any amino acid

<400> 22
Leu Lys Val Leu Val Met Asp Glu Asn Gly Val Ser Arg Met Val Thr
1 5 10 15

Lys Gly Leu Leu Val His Leu Gly Cys Glu Val Thr Thr Val Ser Ser
20 25 30

Asn Glu Glu Cys Leu Arg Val Val Ser His Glu His Lys Val Val Phe
35 40 45

Met Asp Val Cys Met Pro Gly Val Glu Asn Tyr Gln Ile Ala Leu Arg
50 55 60

Ile His Xaa Pro Leu Leu Val Ala Leu Ser Gly Asn Thr Asp Lys Ser
65 70 75 80

Thr Lys Glu Lys Cys Met Ser Phe Gly Leu Asp Gly Val Leu Leu Lys
85 90 95

Pro Val Ser Leu Asp Asn Ile Arg Asp Val Leu Ser Asp Leu Leu
100 105 110

<210> 23
<211> 1716
<212> DNA
<213> Brassica napus

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<222> 673
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antttttattt tnattngttn tactagggac attagtttta acnttttata tatcatgtaa 120

caaaaaaaaa aaaaacnttt tatatntcaa ctatgagcaa ttattcttat agtgttttct 180

ttttccagaa atttgacgac aacctaacta aaacaattta atttgacgtt agttaagtaa 240

tttatataga tggataaatt gagcaagcac attacgaact gcggatcaag gagagtcaca 300

atttaattct tacgttatac acaaaattat ctaaatacta tatatatata cagctgcatg 360

ctacgataat gatcaaatgt ttatgtactt ttcagcgaaa attcttgtcg ccatacatta 420

ctgtgttaat gaatcattaa atatgtgaag gaggaaaaga gtacaaaagg agttttgttg 480

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aggcatttcg cagacactga aatgtgaata ataataaagg aattgccgaa ttgatttcta 540

gttggtgaag tgggtgaaaa ttgtatgtcc attgcttata aactataaaa tataatatnt 600

tnatattatc actntggaca ttagtnngat agaccctagc taaaattttt aaaaattata 660

cattcatttt ctnaagtacc aaacttaatt atcacaatcg gataaaattg tttaagaaac 720

cattacaaac tcagcttgtg gactctgaga gaaactaaga gctagacata cggttagtag 780

tgtagccgca ttttttatgc ttaatttgct taagcatgac ttctatgctc cttgatgata 840

tttattttaa tatcctagga catatggatt tgataaagat cttatcaacc tttcaacaag 900

accattagct caacaaacaa aatactgaaa gtatataatc ttggttacag aattcttatg 960

ccaaaaatat cataatatat atagaattcg gttatgatta agatgaatta tttaattaat 1020

atatttttca cttttgtttt cttatgtatt cttagtattt gttcaccata ttgaccgatt 1080

ggtgtcatat tagtttggta agacaactca gttgcaacga tgcagattac atttcaggaa 1140

gattcatgta agaaagatat ttcgctttgt ggtgtgaaaa tatgcctctt tcactttttt 1200

tcaactataa atttcgatcg atgtatctac gttcttaaca caattcacaa tcttctttag 1260

aatccaaaat tgtaagccgc tttctaatct ctttctcagt atacatatgt aatatgtatg 1320

catatattat tattcataat acaaacacga acccatgcat gcaagaagat agttacacgc 1380

tcataacaaa cacaaaaaaa catacgcatg cattagaaca cttgtatgtt aatttccata 1440

atgttttgca taaacattct tcgttttaat tagcttcttt ttgtgtgaag attgttcgaa 1500

gaaaaaaaac cgaag atg gca aca acg tca aca tcc acg gga gat atc aag   1551
                 Met Ala Thr Thr Ser Thr Ser Thr Gly Asp Ile Lys
                  1               5                   10

aaa acc aag tca gta gaa gtg aag aag aaa ctt aac gtg ttg atc gtt   1599
Lys Thr Lys Ser Val Glu Val Lys Lys Lys Leu Asn Val Leu Ile Val
         15                  20                  25

gat gat gat aca gta att cgt aaa ctt cac gag aat atc atc aaa tcg   1647
Asp Asp Asp Thr Val Ile Arg Lys Leu His Glu Asn Ile Ile Lys Ser
     30                  35                  40

atc ggt gga att tca cag acg gct aag aac ggt gag gag gca gtg aac   1695
Ile Gly Gly Ile Ser Gln Thr Ala Lys Asn Gly Glu Glu Ala Val Asn
 45                  50                  55                  60

atc cac cgc gac ggc aat gca                                       1716
Ile His Arg Asp Gly Asn Ala
                 65
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<210> 24
<211> 67
<212> PRT
<213> Brassica napus

<400> 24
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1   5   10   15

Val Glu Val Lys Lys Lys Leu Asn Val Leu Ile Val Asp Asp Asp Thr
            20   25   30

Val Ile Arg Lys Leu His Glu Asn Ile Ile Lys Ser Ile Gly Gly Ile
        35   40   45

Ser Gln Thr Ala Lys Asn Gly Glu Glu Ala Val Asn Ile His Arg Asp
    50   55   60

Gly Asn Ala
65

<210> 25
<211> 576
<212> DNA
<213> Brassica napus

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<223> n= any nucleotide

<220>
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<223> n= any nucleotide

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gaatttcaca gacagctaag aacggtgagg aggcagtgaa catccaccgc gacggcaatg 120

catctttcga ccttatccta atggataaag aaatgcccga gagggatgga ctttcggcaa 180

ctaagaagct aagagaaatg aaagtgacgt ctatgattat tggggtgacg acactggctg 240

acaatgaaga ggaacgtaag gctttcatgg aagctggact taaccattgc ttggcaaaac 300

ccttaagcaa agccaagatc ctccctctca tcaacaatct catggatgct tgatggatgg 360

atgaattgtc gccactacat atctacatta tacaaatatg aaaaacacat ataataacgt 420

catacacctg tgtgtgtatg catagatatc tatccgcatg tgtgttttta gggttgttat 480

gtttgatttt tattgtgcgt ggcgtgatat acaatcangt nagtcgttac ttttggctta 540

taaaataatg aataagattt gttaaaaata aaaaaa 576

<210> 26
<211> 116
<212> PRT
<213> Brassica napus

<220>
<221> Unsure
<222> 2
<223> Xaa= any amino acid

<400> 26
Val Xaa Asp Asp Pro Val Ile Arg Lys Leu His Glu Ile Ile Ile Lys
1 5 10 15

Ser Ile Gly Gly Ile Ser Gln Thr Ala Lys Asn Gly Glu Glu Ala Val
20 25 30

Asn Ile His Arg Asp Gly Asn Ala Ser Phe Asp Leu Ile Leu Met Asp
35 40 45

Lys Glu Met Pro Glu Arg Asp Gly Leu Ser Ala Thr Lys Lys Leu Arg
50 55 60

Glu Met Lys Val Thr Ser Met Ile Ile Gly Val Thr Thr Leu Ala Asp
65 70 75 80

Asn Glu Glu Glu Arg Lys Ala Phe Met Glu Ala Gly Leu Asn His Cys
85 90 95

Leu Ala Lys Pro Leu Ser Lys Ala Lys Ile Leu Pro Leu Ile Asn Asn
100 105 110

Leu Met Asp Ala
115

<210> 27
<211> 818
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (180)..(605)

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<222> 350
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<220>

<221> Unsure
<222> 57
<223> Xaa= any amino acid

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tgatcacaca cgcacacaca tagaaacata aacacgcaat aatttctata cagtttaatt 120

tcatttttaa cttacttctt ttttttggt gaagattctt gagagaaaag aaatcgaag 179

atg gca aca aaa tcc acc gga ggt acc gag aaa acc aag tcg ata gaa 227
Met Ala Thr Lys Ser Thr Gly Gly Thr Glu Lys Thr Lys Ser Ile Glu
1 5 10 15

gtg aag aag aaa cta atc aac gtg ttg atc gtc gat gat gat cca tta 275
Val Lys Lys Lys Leu Ile Asn Val Leu Ile Val Asp Asp Asp Pro Leu
20 25 30

aac cgt aga ctc cac gag atg atc atc aaa acg atc gga gga att tct 323
Asn Arg Arg Leu His Glu Met Ile Ile Lys Thr Ile Gly Gly Ile Ser
35 40 45

cag act gca aag aat ggc gaa gag gcn gtg atc ctc cac cgt gac ggc 371
Gln Thr Ala Lys Asn Gly Glu Glu Xaa Val Ile Leu His Arg Asp Gly
50 55 60

gaa gca tct ttc gac ctt att cta atg gat aag gaa atg cct gag agg 419
Glu Ala Ser Phe Asp Leu Ile Leu Met Asp Lys Glu Met Pro Glu Arg
65 70 75 80

gat gga gtt tcg aca att aag ang cta aga gaa atg aaa ggg acg tca 467
Asp Gly Val Ser Thr Ile Lys Xaa Leu Arg Glu Met Lys Gly Thr Ser
85 90 95

atg atc gtt ggg gta acg tca gta gct gac caa gaa gaa gag cgt aag 515
Met Ile Val Gly Val Thr Ser Val Ala Asp Gln Glu Glu Glu Arg Lys
100 105 110

gct ttt atg gaa gct ggg ctc aac cat tgc ttg gaa aaa ccc tta acc 563
Ala Phe Met Glu Ala Gly Leu Asn His Cys Leu Glu Lys Pro Leu Thr
115 120 125

aag gcc aag atc ttc ccg ctc att agc cac ctc ttc gat gct 605
Lys Ala Lys Ile Phe Pro Leu Ile Ser His Leu Phe Asp Ala
130 135 140

tgatggatga aggctcatta atgtatctat attttcaatc atgaaatcac ctacacgtgt 665

atttgacaca aaaatctgca tttgttgtga tatagggttt ctcatatcta tgtttgattt 725

attttcttat cgtccgaggt aaaatcatgc aagtcatttc ttttggctaa taaaatatta 785

aaataaggtt ttctcaaaaa aaaaaaaaaa aaa 818

<210> 28
<211> 142
<212> PRT
<213> Arabidopsis thaliana

<220>
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<222> 57
<223> Xaa= any amino acid

<220>
<221> Unsure
<222> 88
<223> Xaa= any amino acid

<400> 28
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  1               5                  10                  15

Val Lys Lys Lys Leu Ile Asn Val Leu Ile Val Asp Asp Asp Pro Leu
             20                  25                  30

Asn Arg Arg Leu His Glu Met Ile Ile Lys Thr Ile Gly Gly Ile Ser
         35                  40                  45

Gln Thr Ala Lys Asn Gly Glu Glu Xaa Val Ile Leu His Arg Asp Gly
     50                  55                  60

Glu Ala Ser Phe Asp Leu Ile Leu Met Asp Lys Glu Met Pro Glu Arg
 65                  70                  75                  80

Asp Gly Val Ser Thr Ile Lys Xaa Leu Arg Glu Met Lys Gly Thr Ser
                 85                  90                  95

Met Ile Val Gly Val Thr Ser Val Ala Asp Gln Glu Glu Glu Arg Lys
            100                 105                 110

Ala Phe Met Glu Ala Gly Leu Asn His Cys Leu Glu Lys Pro Leu Thr
        115                 120                 125

Lys Ala Lys Ile Phe Pro Leu Ile Ser His Leu Phe Asp Ala
    130                 135                 140
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<210> 29
<211> 1324
<212> DNA
<213> Arabidopsis thaliana

<220>
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<222> (1200)..(1322)

<220>
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<222> 1142
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tgggcccaat gggctactaa aacagtttca cgattgtttt tttttttttt tttttaattt 120

ttaacatgta tgtgggatat ttggctataa attatgtaaa aaatttcacg atagattgtt 180

gaatttttga atttcgagtt aaaatatctt caaattacct cacatttaca aaaaggtaga 240

actgttgaaa aactaatgct ctataaaaca ctagacaata acaaaatacg taatgcgtaa 300

agaacctaaa ttatgatttt atttatcttt cttccttttt ccgtgagtat aagccatttt 360

tcatagtaaa gcattacgaa tacgacattg aacactactg acatataaag tagtagattt 420

tgatgggtta acttgtatgc ttaatttgct taagcatgaa cttcaatgct tttataaaag 480

tacttcatga gaatattcct cgttctatac tagcagaagg gttcgatagt gattttacaa 540

ccgttcaaca aaacctttaa acccaaaaaa ccaaagaatg aaagtatcta aacttgatta 600

tacatttctt gtctaaatta tcaaataaca tactctcttt tgtttactta taaacgatat 660

gaaagaaata aataaaaaga acatagaatc ttattatgat ctagaagaat taattaaaga 720

aatatatata tattttttttt catttctact catgtttctt atacattctt taaatttgtt 780

caccattttg atttacttgt tctcatatta gtttgttata caactcactt agaataatgt 840

agattacatt tcagccaaat tcatgtaaaa gatgcttttc tttgtgatgt ttttaaaatg 900

ctttcttttc acttttttttc tttcttaact ataaatcttg atcgaatgcc taccttctta 960

gaacataaga tcttctttaa aatccaaaat cgtaggccac tatttcatta tacttatgta 1020

atatatgtga tacagataca tntatataca aattaaacac gaaaccatac atgcacggtg 1080

tgatcacaca cgcacacaca tagaaacata aacacgcaat aatttctata cagtttaatt 1140

tcatttttaa cttacttctt tttttttggt gaagattctt gagagaaaag aaatcgaag 1199

atg gca aca aaa tcc acc gga ggt acc gag aaa acc aag tcg ata gaa 1247
Met Ala Thr Lys Ser Thr Gly Gly Thr Glu Lys Thr Lys Ser Ile Glu
1 5 10 15

gtg aag aag aaa cta atc aac gtg ttg atc gtc gat gat gat cca tta 1295
Val Lys Lys Lys Leu Ile Asn Val Leu Ile Val Asp Asp Asp Pro Leu
20 25 30

aac cgt aga ctc cac gag tgt cat caa aa 1324
Asn Arg Arg Leu His Glu Cys His Gln
35 40

<210> 30
<211> 41
<212> PRT
<213> Arabidopsis thaliana

<400> 30
Met Ala Thr Lys Ser Thr Gly Gly Thr Glu Lys Thr Lys Ser Ile Glu
1   5   10   15

Val Lys Lys Lys Leu Ile Asn Val Leu Ile Val Asp Asp Asp Pro Leu
            20   25   30

Asn Arg Arg Leu His Glu Cys His Gln
        35   40

<210> 31
<211> 1657
<212> DNA
<213> Brassica napus

<220>
<221> CDS
<222> (145)..(1443)

<400> 31
ggcatcacga gggtacccgt aaatcccacc atacaacaaa gttctgtgaa agtctcccaa 60

aaactgcaaa gagtctcata ttagttctta ctctcagaaa taaaacacac tctttctgaa 120

aagattagcg tttcaaaccc cgaa atg gcc cgt tgt cat gga agt ctt gct 171
Met Ala Arg Cys His Gly Ser Leu Ala
1   5

att ttc tta tgc gtt ctt ttg atg ctc gct tgc tgc caa gct ttg agt 219
Ile Phe Leu Cys Val Leu Leu Met Leu Ala Cys Cys Gln Ala Leu Ser
10   15   20   25

agc aac gta gat gat gga tat ggt cat gaa gat gga agc ttc gaa acc 267
Ser Asn Val Asp Asp Gly Tyr Gly His Glu Asp Gly Ser Phe Glu Thr
                30   35   40

gat agt tta atc aag ctc aac aac gac gac gac gtt ctt acc ttg aaa 315
Asp Ser Leu Ile Lys Leu Asn Asn Asp Asp Asp Val Leu Thr Leu Lys
            45   50   55

agc tcc gat aga ccc act acc gaa tca tca act gtt agt gtt tcg aac 363
Ser Ser Asp Arg Pro Thr Thr Glu Ser Ser Thr Val Ser Val Ser Asn
        60   65   70

ttc gga gca aaa ggt gat gga aaa acc gat gat act cag gct ttc aag 411
Phe Gly Ala Lys Gly Asp Gly Lys Thr Asp Asp Thr Gln Ala Phe Lys
    75   80   85

aaa gca tgg aag aag gca tgt tca aca aat gga gtg act act ttc ttg 459

Lys Ala Trp Lys Lys Ala Cys Ser Thr Asn Gly Val Thr Thr Phe Leu
90 95 100 105

att cct aaa ggg aag act tat ctc ctt aag tct att aga ttc aga ggc 507
Ile Pro Lys Gly Lys Thr Tyr Leu Leu Lys Ser Ile Arg Phe Arg Gly
110 115 120

cca tgc aaa tca tta cgt agc ttc cag atc cta ggc act tta tca gct 555
Pro Cys Lys Ser Leu Arg Ser Phe Gln Ile Leu Gly Thr Leu Ser Ala
125 130 135

tct aca aaa cga tcg gat tac agt aat gac aag aac cac tgg ctt att 603
Ser Thr Lys Arg Ser Asp Tyr Ser Asn Asp Lys Asn His Trp Leu Ile
140 145 150

ttg gag gac gtt aat aat cta tca atc gat ggc ggc tcg gcg ggg att 651
Leu Glu Asp Val Asn Asn Leu Ser Ile Asp Gly Gly Ser Ala Gly Ile
155 160 165

gtt gat ggc aac gga aaa atc tgg tgg caa aac tca tgc aaa atc gac 699
Val Asp Gly Asn Gly Lys Ile Trp Trp Gln Asn Ser Cys Lys Ile Asp
170 175 180 185

aaa tct aag cca tgc aca aaa gcg cca acg gct ctt act ctc tac aac 747
Lys Ser Lys Pro Cys Thr Lys Ala Pro Thr Ala Leu Thr Leu Tyr Asn
190 195 200

cta aac aat ttg aat gtg aag aat ctg aga gtg aga aat gca cag cag 795
Leu Asn Asn Leu Asn Val Lys Asn Leu Arg Val Arg Asn Ala Gln Gln
205 210 215

att cag att tcg att gag aaa tgc aac agt gtt gat gtt aag aat gtt 843
Ile Gln Ile Ser Ile Glu Lys Cys Asn Ser Val Asp Val Lys Asn Val
220 225 230

aag atc act gct cct ggc gat agt ccc aac acg gat ggt att cat atc 891
Lys Ile Thr Ala Pro Gly Asp Ser Pro Asn Thr Asp Gly Ile His Ile
235 240 245

gtt gct act aaa aac att cga atc tcc aat tca gac att ggg aca ggt 939
Val Ala Thr Lys Asn Ile Arg Ile Ser Asn Ser Asp Ile Gly Thr Gly
250 255 260 265

gat gat tgc ata tcc att gag gat gga tcg caa aat gtt caa atc aat 987
Asp Asp Cys Ile Ser Ile Glu Asp Gly Ser Gln Asn Val Gln Ile Asn
270 275 280

gat tta act tgc ggc ccc ggt cat ggc atc agc att gga agc ttg ggg 1035
Asp Leu Thr Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser Leu Gly
285 290 295

gat gac aat tcc aaa gct tat gta tcg gga att aat gtg gat ggt gct 1083
Asp Asp Asn Ser Lys Ala Tyr Val Ser Gly Ile Asn Val Asp Gly Ala
300 305 310

acg ctc tct gag act gac aat gga gta aga atc aag act tac cag gga 1131

Thr Leu Ser Glu Thr Asp Asn Gly Val Arg Ile Lys Thr Tyr Gln Gly
    315   320   325

ggg tca gga act gct aag aac att aaa ttc caa aac att cgt atg gat   1179
Gly Ser Gly Thr Ala Lys Asn Ile Lys Phe Gln Asn Ile Arg Met Asp
330   335   340   345

aat gtc aag aat ccg atc ata atc gac cag aac tac tgc gac aag gac   1227
Asn Val Lys Asn Pro Ile Ile Ile Asp Gln Asn Tyr Cys Asp Lys Asp
                350   355   360

aaa tgc gaa caa caa gaa tct gcg gtt caa gtg aac aat gtc gtg tat   1275
Lys Cys Glu Gln Gln Glu Ser Ala Val Gln Val Asn Asn Val Val Tyr
            365   370   375

cgg aac ata caa ggt acg agc gca acg gat gtg gcg ata atg ttt aat   1323
Arg Asn Ile Gln Gly Thr Ser Ala Thr Asp Val Ala Ile Met Phe Asn
        380   385   390

tgc agt gtg aaa tat cca tgc caa ggt att gtg ctt gag aat gtg aac   1371
Cys Ser Val Lys Tyr Pro Cys Gln Gly Ile Val Leu Glu Asn Val Asn
    395   400   405

atc aaa gga gga aaa gct tct tgc aaa aat gtc aat gtt aag gat aaa   1419
Ile Lys Gly Gly Lys Ala Ser Cys Lys Asn Val Asn Val Lys Asp Lys
410   415   420   425

ggc acc gtt tct cct aaa tgc cct taattactaa gttgattatg taatatacat   1473
Gly Thr Val Ser Pro Lys Cys Pro
                430

aaatacgtat tatatgtggt tatagatgcc atctatatcc ttatctacgt attgattctc 1533

gatatatata gaaaactaag gatttatggg aatatacata caatagttga gataattgtt 1593

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Gly His Glu Asp Gly Ser Phe Glu Thr Asp Ser Leu Ile Lys Leu Asn
         35   40   45

Asn Asp Asp Asp Val Leu Thr Leu Lys Ser Ser Asp Arg Pro Thr Thr

50 55 60

Glu Ser Ser Thr Val Ser Val Ser Asn Phe Gly Ala Lys Gly Asp Gly
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Lys Thr Asp Asp Thr Gln Ala Phe Lys Lys Ala Trp Lys Lys Ala Cys
85 90 95

Ser Thr Asn Gly Val Thr Thr Phe Leu Ile Pro Lys Gly Lys Thr Tyr
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Leu Leu Lys Ser Ile Arg Phe Arg Gly Pro Cys Lys Ser Leu Arg Ser
115 120 125

Phe Gln Ile Leu Gly Thr Leu Ser Ala Ser Thr Lys Arg Ser Asp Tyr
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Ser Asn Asp Lys Asn His Trp Leu Ile Leu Glu Asp Val Asn Asn Leu
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Ser Ile Asp Gly Gly Ser Ala Gly Ile Val Asp Gly Asn Gly Lys Ile
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Trp Trp Gln Asn Ser Cys Lys Ile Asp Lys Ser Lys Pro Cys Thr Lys
180 185 190

Ala Pro Thr Ala Leu Thr Leu Tyr Asn Leu Asn Asn Leu Asn Val Lys
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Asn Leu Arg Val Arg Asn Ala Gln Gln Ile Gln Ile Ser Ile Glu Lys
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Cys Asn Ser Val Asp Val Lys Asn Val Lys Ile Thr Ala Pro Gly Asp
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Ser Pro Asn Thr Asp Gly Ile His Ile Val Ala Thr Lys Asn Ile Arg
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290 295 300

Val Ser Gly Ile Asn Val Asp Gly Ala Thr Leu Ser Glu Thr Asp Asn
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Gly Gly Leu Glu Thr Val Lys Val Lys Leu Pro Ala Val Ile Ser Ala
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gac ttg cgg ctc aat gag ccg cgg tac gct act ctg ccc aat atc atg 143
Asp Leu Arg Leu Asn Glu Pro Arg Tyr Ala Thr Leu Pro Asn Ile Met
35 40 45

aag gcc aag aag aag ccc atc aaa aag ctc aca gcc aca gat gtc ggt 191
Lys Ala Lys Lys Lys Pro Ile Lys Lys Leu Thr Ala Thr Asp Val Gly
50 55 60

gtg gac ttg gcg cca cgt caa caa gtg ttg agc gta gaa gac ccg ccc 239
Val Asp Leu Ala Pro Arg Gln Gln Val Leu Ser Val Glu Asp Pro Pro
65 70 75

acc aga cag gct ggt tcc att gtg cct gat gtc gac act ctc atc acc 287
Thr Arg Gln Ala Gly Ser Ile Val Pro Asp Val Asp Thr Leu Ile Thr
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Lys Leu Lys Glu Lys Gly His Leu
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Ala Lys Lys Lys Pro Ile Lys Lys Leu Thr Ala Thr Asp Val Gly Val
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Asp Leu Ala Pro Arg Gln Gln Val Leu Ser Val Glu Asp Pro Pro Thr
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Lys Ala Glu Val Gln Met Lys Leu Val Pro Gly Asn Ser Ala Gly Thr
35 40 45

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Val Thr Thr Phe Tyr Leu Lys Ser Pro Gly Thr Thr Trp Asp Glu Ile
50 55 60

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65 70 75

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35 40 45

Thr Thr Phe Tyr Leu Lys Ser Pro Gly Thr Thr Trp Asp Glu Ile Asp
50 55 60

Phe Glu Phe Leu Gly Asn Ile Ser Gly His Pro Tyr Thr Leu His Thr
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534 Rec'd PCT/PTO 20 SEP 2000

~~SIGNAL TRANSDUCTION PROTEIN INVOLVED~~ IN PLANT DEHISCENCE

This invention relates to novel plant nucleic acid sequences and proteins. The sequences and proteins are useful in the control of plant dehiscence and in the production of male sterile plants.

The production of seed is an important developmental process in all higher plants. In oilseed rape (*Brassica napus*), following abscission of floral parts, pods or siliques are formed which contain 15-30 seeds. Around 50-70 days after anthesis (DAA) the pods become susceptible to shatter, a process that serves to expel the mature seeds into the surrounding environment. In the days leading to dehiscence, an array of anatomical, molecular and biochemical changes take place, thus preparing both seed and pod for the event. Shatter eventually occurs as a result of a combination of factors including: the creation of tensions within the pod between the lignified valve edge cells of the endocarp and the unlignified dehiscence zone (DZ) cells, weakening of the DZ cell walls by hydrolytic enzyme activity and ultimately due to physical forces such as strong winds or harvesting machinery.

Pod development in *B. napus* can be segmented into three stages. In the first stage, which occurs 0-20 DAA, the newly formed siliques, consisting of two seed-containing carpels separated by a false septum and a replar region, grow to their full length of around 10cm. The seeds begin to grow when the pods are virtually full size [Hocking and Mason, 1993]. Between 10 and 20 DAA the cells in the replar region begin to differentiate into replar cells, large valve edge cells and form a distinct region, 1-3 cells wide, comprising the DZ [Meakin and Roberts, 1990a].

The second stage occurs between 20 and 50 DAA. From 20 DAA, in conjunction

with termination of pod elongation, secondary cell wall material is deposited in the valve edge cells, and the replar cells become increasingly lignified. The DZ cells do not exhibit thickening of the cell wall. A progressive shrinkage and loss of organelles is apparent in the DZ cells from 40 DAA onwards and eventually these cells separate completely due to hydrolysis of the middle lamella [Meakin and Roberts, 1990a]. In the final stage of pod development, which occurs 50-70 DAA, the lignified cells undergo senescence and the necessary tensions are created so that the desiccated pod, containing mature seed, eventually shatters.

Molecular studies of the penultimate stage of pod development have revealed a spatial and temporal correlation between the up-regulation of a number of mRNAs and pod dehiscence in *B. napus*. These mRNAs encode a polygalacturonase (PG) and a proline-rich protein (SAC51). Further analysis of the expression of the PG following fusion of a pod-specific *Arabidopsis thaliana* PG promoter to GUS [Jenkins et al., (1997)], has revealed that reporter gene expression is restricted precisely to the layer of cells comprising the pod DZ in transgenic *B. napus*. From 40 DAA, Meakin and Roberts (1990b) reported a progressive increase in β-1,4-glucanase (cellulase) activity in the DZ.

It is understood that the processes of dehiscence and abscission are not regulated by the same environmental or chemical signals, but that they involve controlled degradation of cell wall material and cell separation in a distinct group of cells. Both ethylene and indole-3-acetic acid (IAA) appear to be important regulators of the timing of the abscission process but the role of these plant hormones in dehiscence is less clearly defined. The increase in cellulase activity has been shown to correlate with a rise in the production of ethylene, mainly from the seed, which peaks at around 40 DAA [Meakin and Roberts, 1990b; Johnson-Flanagan and Spencer, 1994].

Developmental processes, such as pod dehiscence, which involve highly regulated and controlled expression of an array of different genes at a precise time and cellular location, clearly require an intricate signal transduction network.

Further and improved genetic elements to control plant processes in this area are constantly desired. We describe the isolation, for the first time, of a plant cDNA (DZ2) encoding an individual response regulator protein, the expression of which is closely correlated with dehiscence of fruit in *B. napus*. DZ2 has a role in the ability to control molecule signaling during the events leading to shatter and thus to control pod shatter in plants. In addition to the identification of the nucleic acid termed "DZ2" a homologous, but not identical sequence and protein were also identified from *B. napus*. This sequence was designated "DZ2B". Sequence analysis of DZ2 and DZ2B shows that there are two DZ2 genes in *B. napus*, each represented by a slightly different cDNA (here termed DZ2 and DZ2B). This is consistent with one gene being encoded by the *B. campestris* derived genome and the other from the genome derived from *B. oleracea*. In this text, the designation "DZ2" is equivalent to the CW1 designation in UK 9806113.8 (as seen from Figure 1).

According to a first aspect of the invention there is provided nucleic acid optionally encoding a signal transduction protein involved in the process of dehiscence. Such a sequence or signal transduction protein has never previously been described in plant dehiscence.

In this text, the term "involved in the process of dehiscence" means any nucleic acid (preferably) encoding any protein which has an effect in the dehiscence process, in particular a protein or nucleic acid sequence involved in an MAP Kinase cascade or any other protein or nucleic acid sequence which results in changes in the expression of genes involved in dehiscence, such as upregulation of genes encoding

polygalacturonase, cellulase, senescence-related proteins and/or downregulation of genes encoding for proteins involved in cells wall biosynthesis. The nucleic acid sequences/proteins of the present invention which are "involved in the process of plant dehiscence" are not the individual structural genes or proteins which cause dehiscence (polygalacturonases etc.). Rather, the nucleic acid sequences/proteins of the present invention are sequences/proteins which have an effect on the expression of such structural genes or proteins. One advantage of the present invention is that the use of such nucleic acid sequences/proteins enables the possibility to influence the whole process of dehiscence rather than just one element of it. Preferably the protein or nucleic acid sequence of the present invention which is involved in the process of dehiscence effects a structural protein which is a hydrolytic enzyme such as polygalacturonase or cellulase.

The nucleic acid of the first aspect of the invention may be a nucleic acid which is naturally expressed in a dehiscence zone. Such a nucleic acid will most accurately reflect nucleic acid naturally expressed in a plant. Preferably the dehiscence zone is a pod (also termed "siliques"), anther and/or funiculus dehiscence zone. Preferably the plant is a member of the Brassica family, maize, wheat, soyabean, *Cuphea* or sesame.

A second aspect of the invention provides nucleic acid encoding a protein wherein the protein:

a) comprises the amino acid sequence shown in figure 1 or;

b) has one or more amino acid deletions, insertions or substitutions relative to a protein as defined in a) above, but has at least 40% amino acid sequence identical therewith; or

c) is a fragment of a protein as defined in a) or b) above, which is at least 10 (preferably 20 or 30) amino acids long.

The percentage amino acid identity can be determined using the default parameters of the GAP computer program, version 6.0 described by Deveraux *et al.,* 1984 and available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilises the alignment method of Needleman and Wunsch 1970 as revised by Smith and Waterman 1981. More preferably the protein has at least 45% identity to the amino acid sequence of Figure 1, through 50%, 55% 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% identity using the default parameters.

The skilled person will appreciate that various changes can sometimes be made to the amino acid sequence of a protein (which has a desired property) to produce variants (often known as "muteins") which still have said property. Such variants of the protein describe in a, b and c above are within the scope of the present invention and are discussed in greater detail below in sections *(i)* to *(iii)*. They include allelic and non-allelic variants.

(i) Substitutions

An example of a variant of the present invention is a polypeptide as defined in a, b or c above, apart from the substitution of one or more amino acids with one or more other amino acids.

The skilled person is aware that various amino acids have similar characteristics. One or more such amino acids of a protein can often be substituted by one or more other such amino acids without eliminating a desired property of that protein.

For example, the amino acids glycine, alanine, valine, leucine and isoleucine can often be substituted for one another (amino acids having aliphatic side chains). Of these possible substitutions it is preferred that glycine and alanine are used to substitute for one another (since they have relatively short side chains) and that valine, leucine and isoleucine are used to substitute for one another (since they have larger aliphatic side chains which are hydrophobic). Other amino acids that can often be substituted for one another include phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains); lysine, arginine and histidine (amino acids having basic side chains); aspartate and glutamate (amino acids having acidic side chains); asparagine and glutamine (amino acids having amide side chains); and cysteine and methionine (amino acids having sulphur containing side chains).

Substitutions of this nature are often referred to as "conservative" or "semi-conservative" amino acid substitutions.

(ii) Deletions

Amino acid deletions can be advantageous since the overall length and the molecular weight of a polypeptide can be reduced whilst still retaining a desired property. This can enable the amount of protein required for a particular purpose to be reduced. Proteins according to the present invention, which have such deletion(s) are useful. They may interfere with the normal functioning of DZ2; that is, they may act as dominant negative mutations preventing normal DZ2 functioning and thus be of particular value, for example, in reducing pod shatter.

The amino acid sequence shown in figure 1 has various functional regions. For particular applications of the present invention, one or more of these regions may not be needed and may therefore be deleted.

(iii) Insertions

Amino acid insertions relative to a polypeptide as defined in a, b or c above can also be made. This may be done to alter the nature of the protein (e.g. to assist in identification, purification, or expression, as explained below in relation to fusion proteins).

Changes in the protein according to the present invention can produce versions of the protein that are constitutively active. If a protein of the present invention acts on an inhibitor of the release of hydrolytic enzymes, then a constitutively active version would prevent or reduce pod shatter

A protein according to any aspect of the invention may have additional N-terminal and/or C-terminal amino acid sequences. Such sequences can be provided for various reasons. Techniques for providing such sequences are well known in the art. They include using gene-cloning techniques to ligate together nucleic acid molecules encoding polypeptides or parts thereof, followed by expressing a polypeptide encoded by the nucleic acid molecule produced by ligation.

Additional sequences may be provided in order to alter the characteristics of a particular polypeptide. This can be useful in improving expression or regulation of expression in particular expression systems. For example, an additional sequence may provide some protection against proteolytic cleavage. This has been done for the hormone somatostatin by fusing it at its N-terminus to part of the β galactosidase enzyme [Itakwa *et al.*, 105-63 (1977)].

Additional sequences can also be useful in altering the properties of a polypeptide to aid in identification or purification.

For example, a signal sequence may be present to direct the transport of the polypeptide to a particular location within a cell or to export the polypeptide from the cell. Hydrophobic sequences may be provided to anchor a polypeptide in a membrane. Thus the present invention includes within its scope both soluble and membrane-bound polypeptides.

Preferably, the nucleic acid according to the second aspect of the invention encodes a signal transduction protein or a functional portion thereof involved in the process of dehiscence. All preferred features of the first aspect of the invention as described above also apply to the second.

The term protein used in this text means, in general terms, a plurality of amino acid residues joined together by peptide bonds. It is used interchangeably and means the same as polypeptide or peptide.

The nucleic acid according to the first or second aspect of the invention preferably comprises the sequence set out in figure 1 or a sequence which is 40% or more identical, preferably through 45%, 50%, 55%, 60%, 65%,70%, 75%, 80%, 85%, 90%, 95% to the sequence in Figure 1 at the nucleic acid residue level, using the default parameters of the GAP computer program, version 6.0 described by Deveraux *et al.*, 1984 and available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilises the alignment method of Needleman and Wunsch 1970 as revised by Smith and Waterman 1981. Further, the nucleic acid may comprise a fragment of a sequence according to the first or second aspect which is at least 30 bases long also 40, 50, 60, 70, 80, or 90 bases in length. While this nucleic acid is the preferred nucleic acid of the invention, it is well known to those persons skilled in the art that because of the nucleic acid "degenerate code" which encodes nucleic acids, a considerable number of variations in nucleic acid

sequence can be used to encode for proteins according to the first or second aspects of the invention.

The nucleic acid of the first or second aspects of the invention may be isolated or recombinant and may be in substantially pure form. The nucleic acid may be antisense to nucleic acid according to the first or second aspects of the invention. As understood by the person skilled in the art introducing the coding region of a gene in the reverse orientation to that found in nature (antisense) can result in the downregulation of the gene and hence the production of less or none of the gene product. The transcribed antisense DNA is capable of binding to and destroying the function of the sense RNA of the sequence normally found in the cell, thereby disrupting function. Antisense nucleic acid may be constitutively expressed, but is preferably limited to expression in those zones (dehiscence) in which the naturally occurring nucleic acid is expressed.

The nucleic acid according to the first or second aspects of the invention preferably include a promoter or other regulatory sequence which controls expression of the nucleic acid. Promoters and other regulatory sequences which control expression of a nucleic acid in dehiscence zones are known in the art, for example described in WO96/30529 and WO94/23043. Further promoters or other regulatory sequences can be identified and can also include the promoter or other regulatory sequence which controls expression of a nucleic acid as set out in figure 1. The person skilled in the art will know that it may not be necessary to utilize the whole promoter or other regulatory sequence. Only the minimum essential regulatory elements may be required and in fact such elements can be used to construct chimeric sequences or promoters. The essential requirement is, of course, to retain the tissue and/or temporal specificity.

The nucleic acid according to the first or second aspects of the invention may be in the form of a vector. The vector may be a plasmid, cosmid or phage. Vectors frequently include one or more expressed markers which enable selection of cells transfected (or transformed: the terms are used interchangeably in this text) with them and preferably, to enable a selection of cells containing vectors incorporating heterologous DNA. A suitable start and stop signal will generally be present and if the vector is intended for expression, sufficient regulatory sequences to drive expression will be present. Nucleic acid according to the first and second aspects of the invention is preferably for expression in plant cells and thus microbial host expression is perhaps less important although not ruled out. Microbial host expression and vectors not including regulatory sequences are useful as cloning vectors.

A third aspect of the invention relates to a cell comprising nucleic acid according to the first or second aspects of the invention. The cell may be termed as "a host" which is useful for manipulation of the nucleic acid, including cloning. Alternatively, the cell may be a cell in which to obtain expression of the nucleic acid, most preferably a plant cell. The nucleic acid can be incorporated by standard techniques known in the art in to cells. Preferably nucleic acid is transformed in to plant cells using a disarmed Ti plasmid vector and carried by an Agrobacterium by procedures known in the art, for example as described in EP-A-0116718 and EP-A-0270822. Foreign nucleic acid can alternatively be introduced directly into plant cells using an electrical discharged apparatus or by any other method that provides for the stable incorporation of the nucleic acid into the cell. Preferably the stable incorporation of the nucleic acid is within the nucleic DNA of any cell preferably a plant cell. Nucleic acid according to the first and second aspects of the invention preferably contains a second "marker" gene that enables identification of the nucleic acid. This is most commonly used to distinguish the transformed plant cell

containing the foreign nucleic acid from other plants cells that do not contain the foreign nucleic acid. Examples of such marker genes include antibiotic resistance, herbicide resistance and Glucuronidase (GUS) expression. Expression of the marker gene is preferably controlled by a second promoter which allows expression of the marker gene in cells other than those than dehiscence zones (if this is the tissue specific expression of the nucleic acid according to the first or second aspects of the invention). Preferably the cell is from any of the Brassica family (most preferably *B. napus)*, maize, wheat, soyabean, *Cuphea* and sesame.

A third aspect of the invention includes a process for obtaining a cell comprising nucleic acid according to the first or second aspects of the invention. The process involves introducing said nucleic acid into a suitable cell and optionally growing on or culturing said cell.

A fourth aspect of the invention provides a plant or a part thereof comprising a cell according to the third aspect of the invention. A whole plant can be regenerated from the single transformed plant cell by procedures well known in the art. The invention also provides for propagating material or a seed comprising a cell according to the third aspect of the invention. The invention also relates to any plant or part thereof including propagating material or a seed derived from any aspect of the invention. The fourth aspect of the invention also includes a process for obtaining a plant or plant part (including propagating material or seed, the process comprising obtaining a cell according to the third aspect of the invention or, indeed, plant material according to the fourth aspect of the invention and growth (to the required plant, plant part, propagating material etc). Techniques for such a process are commonplace in the art.

A fifth aspect of the invention provides a signal transduction protein involved in the

process of the plant dehiscence. The signal transduction protein according to the fifth aspect may have one or more of the preferred features according to the first or second aspects of the invention. Preferably it may be isolated, recombinant or in substantially pure form. It may comprise the various changes according to the first or second aspects. Preferably the protein is expressed from nucleic acid according to the first or second aspects. Alternatively, the protein can be provided using suitable techniques known in the art.

A sixth aspect of the invention provides a protein which;

a) comprises the amino acid sequence shown in figure 1 or;

b) has one or more amino acid deletions, insertions, or substitutions relative to a protein as defined in a) above and has at least 40% amino acid sequence identity therewith;

or a fragment of a protein as defined in a) or b) above which is at least 10 amino acids long. The percentage amino acid identity can be determined using the default parameters of the GAP computer program, version 6.0 described by Deveraux *et al.*, 1984 and available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilises the alignment method of Needleman and Wunsch 1970 as revised by Smith and Waterman 1981. More preferably the protein has at least 45% identity to the amino acid sequence of Figure 1, through 50%, 55% 60%, 65%, 70%, 75% 80%, 85%, 90%, 95% identity using the default parameters.

The protein is preferably a signal transduction protein involved in the process of plant dehiscence and again, the preferred features of aspects one, two and five also applied to the sixth aspect.

The seventh aspect of the invention provides a process for regulating/controlling dehiscence in plant or in a part thereof, the process comprising obtaining a plant or a part thereof according to the fourth aspect of the invention. The process of dehiscence can be regulated and/or controlled by increasing or decreasing the expression of nucleic acid sequences according to the first or second aspect of the invention. Increased or decreased expression can easily be influenced by the person skilled in the art using technology well known. This includes increasing the numbers of copies of nucleic acid according to the invention in a plant or a plant thereof or increasing expression levels of copies of the nucleic acid present in particular parts or zones of the plant. Preferably the zones are dehiscence zones.

The process according to the seventh aspect of the invention includes obtaining a plant cell according to the third aspect of the invention or part of a plant according to the fourth aspect in the invention and deriving a plant therefrom. Alternatively, the process may comprise obtaining propagating material or a seed according to the fourth aspect of the invention and deriving a plant therefrom.

Preferably, the process of the seventh aspect of the invention is in the pod or the anther of a plant. All preferred features of aspects one to six also apply to the seventh.

An eighth aspect of the invention provides for the use of nucleic acid according to the first to seventh aspects of the invention in the regulation/control of plant dehiscence. All preferred features of aspects one to seven also applies to the eighth.

The ninth aspect of the invention provides for the use of nucleic acid according to the first or second aspect of the invention as a probe. Such a probe can be used in techniques well known in the art to identify the presence of identical or homologous

nucleic acid sequences from any source, preferably a plant source. The ninth aspect of the invention also provides nucleic acid identified by use of the nucleic acid from aspects one or two as a probe.

A tenth aspect of the invention provides for the use of nucleic acid according to aspects one or two of the invention in the production of a cell, tissue, plant or part thereof, or propagating material. Again, all preferred features of aspects one and two also apply to the tenth.

An eleventh aspect of the invention provides for nucleic acid comprising one or more of the underlined sequences as set out in figure 1 or the primer sequences in Fig 5, Fig 9 or Fig 11. Such nucleic acid sequences are preferably used as primers in an PCR (Polymerase Chain Reaction) process in order to amplify nucleic acid sequences.

A twelfth aspect of the invention provides the use of nucleic acid according to the first or second aspects of the invention to identify another other protein or proteins which interact with its expression product. Such use can be carried out by the yeast two hybrid screening method (or others known in the art). The yeast two hybrid screening method is described for this aspect of the invention, in general, with reference to the sequence described as DZ2. A potential way to implement the yeast 2-hybrid screen is outlined, as follows:

DZ2 is linked to the Gal4 DNA binding domain and expressed in yeast which contains a pGAL4-lacZ gene. For activity of lacZ a second protein is required that contains the DNA transcriptional activation domain of GAL4 and that interacts with the DZ2 protein. This is provided by making a cDNA expression library from plant DZ zones which results in fusions of plant

proteins to the GAL4 activation domain. This library is transformed into the yeast strain that contains pGAL4-LacZ and expresses the DZ2-Gal4 DNA bining domain protein fusion. Colonies that have lacZ activity are transformed with a gene for a protein that interacts with DZ2.

Using such a system, upstream and downstream components of any signal transduction pathway can be identified, thus resulting in further ability to control/regulate dehiscence and/or male sterility.

A thirteenth aspect of the invention provides for a protein, as defined according to the limitations of the second aspect of the invention (without reference to figure 1) and nucleic acid encoding the protein, wherein the protein is capable of being identified according to the use (or method) according to the twelfth aspect of the invention.

A fourteenth aspect of the invention provides for the use of a protein according to the fifth or sixth aspect of the invention as a probe. In this context the probe is a means to identifying interacting entities (such as other proteins), including upstream and downstream interacting signal components. A protein according to the fifth or sixth aspect of the invention can be used as a probe to directly look for interactions with other proteins, i.e. purified protein can be used to look for complex formation with other plant protein, particularly isolated from the DZ zone. For example, a modified recombinant DZ2 protein can be made with a sequence tag, such as a His-tag, that enables the DZ2 + interacting protein to be directly purified on a His affinity column. Alternatively, an antibody can be raised to DZ2 protein. This antibody is then used to identify DZ2 protein complexes and to purify the complexes. The DZ2 interacting proteins can be purified and microsequenced to enable cloning of the genes for these interacting proteins.

The present invention provides a particularly useful method by which plant dehiscence can be regulated/controlled.

In addition to the use of the present invention in the production of shatter resistance or shatter-delayed plants such as oil seed rape, the invention may be used to control/regulate pollen release (by the control/regulation of anther dehiscence) which can produce male sterile plants. The temporal and spatial expression of nucleic acid encoding a protein according to the first and second aspects of the invention may require adjustment in obtaining the correct levels of dehiscence delay or prevention in different zones. For example, if pod dehiscence is required but anther dehiscence is not, it is necessary to ensure that expression of nucleic acid according to a first or second aspect of the invention has the correct temporal and spatial expression in order to obtain pod dehiscence or delay but not, to any substantial extent, anther dehiscence. This can be obtained by processes known in the art and may require use of particular promoter sequences to obtain the desired result. Usually in plant transformation, some difference in the level of expression of nucleic acid is observed in different plants. In some cases, the ratio of expression levels in different tissues can vary between different plant transformants thus providing essentially tissue-specific expression in one or other of the target tissues in some of the plant transformants. In the present invention, the natural expression of nucleic acid according to the first or second aspects may be predominantly higher in pod dehiscence zones and lower in the anther and funiculus dehiscence zones. However, as described above, it is possible to obtain plants in which the protein expression is regulated in a particular dehiscence zone. Accordingly, a particularly useful aspect of the invention is the provision of plants which have one or both of the following features; are male sterile, are shatter resistant.

As described earlier, the process of dehiscence at the dehiscence zone involves the secretion of a number of enzymes, including hydrolytic enzymes. While previous attempts have been made to down or up regulate specific genes encoding particular proteins involved in the process of dehiscence, regulation by means of a signal transduction protein which effects expression of a number of genes is likely to be more effective than regulation of a single gene. In addition to this, the nucleic acid of the present invention has been identified as being expressed earlier than several other known genes involved in the process of plant dehiscence. This suggests that it is important earlier on in the process of plant dehiscence and can be used to control/regulate plant dehiscence at an earlier stage.

The nucleic acid encoding a signal transduction protein involved in the process of dehiscence or the signal transduction protein itself may be a component of a signal pathway that may either positively or negatively regulate pod shatter.

A more detailed explanation of such regulations/control, described with reference to a pod shatter (dehiscence) model is described below. As a skilled person will acknowledge, the model described below also relates to other general processes of dehiscence such as in the anther.

In the process of dehiscence, a particular signal transduction protein may be required to transmit a signal from the almost mature seed which initiates the expression or release of enzymes required for pod shatter. In this model, developmental signals switch on expression and/or activation of a particular signal transduction protein in the pod dehiscence zone. This leads to expression of genes required for the release of pod dehiscence zone enzymes (such as hydrolytic enzymes). In this case, prevention of activity of the signal transduction protein, for example by downregulation of expression of this protein, would result in reduced dehiscence.

Alternatively, the developing seed may transmit a signal which represses the expression and/or activity of a particular signal transduction protein until late in cell development. In this model, developmental signals switch on a particular signal transduction protein which, in due course, represses the expression of genes required for release of dehiscence zone specific enzymes (such as hydrolytic enzymes). In this case, expression of a modified signal transduction protein that is constitutively active would result in reduced dehiscence.

A signal transduction protein which is either positively or negatively involved in the process of dehiscence can be used according to the present invention.

In addition to DZ2 several other DZ-expressed genes have been previously isolated and individually downregulated to result in *B. napus* plants that have increased resistance to pod shatter; namely Sac66 (WO 96/30529 - Figure 15), DZ15 (Figure 16) and OSR 7(9) (Figure 17). It is anticipated that downregulation of more than one gene involved in pod shatter will further increase resistance to pod shatter. This could be achieved by combining different transgenes by transformation with several transgenes each designed to downregulate a different DZ-expressed gene or by crossing together *B. napus* lines that individually are transformed with such transgenes. Such methods are complex either involving transformation with a construct containing multiple chimeric genes or require the maintenance of several transgenic loci in the breeding program. A preferred method is to transform with a chimeric gene consisting of a single promoter driving expression of an antisense or partial sense transcript which is comprised of elements of all the DZ-expressed genes to downregulated. Similarly a single promoter could be used to drive the expression of multiple ribozymes each targeted against a different DZ-expressed gene. The use of a single promoter to drive expression of a combination of antisense, partial sense

and ribozymes is also possible. Ideally the promoter will be pod DZ-specific, however a useful promoter may be pod-specific or even constitutively active. A suitable DZ-specific promoter would be that of DZ2, DZ2B, DZ2AT3 or ESJ2A (WO 99/13089).

Accordingly, the present invention provides a nucleic acid sequence according to the first or second aspects (and also all aspects which include the first or second aspects) in combination with one or more further nucleic acid sequences which are dehiscence-zone expressed. Examples of such sequences include Sac66, DZ15 and OSR(7), Figures 15-17 respectively. Such sequence may be in sense or in antisense orientation. Such a sequence may be included as full length genomic, full-length cDNA or partial sequences; the sequences may be as shown in the figures or may have the same sequence identity (both for aminoacid sequence and nucleic acid sequence) as described above for the protein according to the second aspect of the invention or the nucleic acid according to the first or second aspects of the invention. As will be recognised by those skilled in the art a partial sequence may be useful in either the sense or antisense orientation.

The invention is described by reference to the enclosed drawings;

Figure 1	DZ2 full length sequence showing original PCR product and primer sites
Figure 2	Amino acid alignment with bacterial response regulator proteins & *ETR1*

Figure 3	Northern analysis of expression of DZ2 in pods and other tissues. The lower panel shows the ethidium bromide-stained RNA gel prior to blotting and probing with DZ2
Figure 4	Comparison of bacterial two-component systems with DZ2
Figure 5	Sequence of the promoter region of B.napus DZ2B.
Figure 6	Nucleic and putative peptide sequence alignments of DZ2 with DZ2B.
Figure 7	Northern analysis of expression of DZ2B in pods and other tissues. The probe was labeled DZ2B cDNA.
Figure 8	Schematic diagram of pDZ2B-GUS-SCV
Figure 9	DZ2AT3 cDNA sequence showing the putative DZ2AT3 peptide.
Figure 10	Amino acid alignment of DZ2AT3 with DZ2 and DZ2B.
Figure 11	Sequence of the promoter region of A.thaliana DZ2AT3.
Figure 12	Schematic diagram of pDZ2AT3-GUS-SCV.
Figure 13	Schematic diagram of pPGL-DZ2as-SCV and pDZ2B-DZ2as-SCV.
Figure 14	Schematic diagram of pWP357-SCV.
Table 1	Pod shatter resistance of WP357-SCV transformants.

Figure 15 Nucleic acid sequence and putative amino acid sequence of Sac66.

Figure 16 Nucleic acid sequence and putative amino acid sequence of DZ15.

Figure 17 Nucleic acid sequence and putative amino acid sequence of OSR7 (9)

The present invention is now described with reference to the following, non-limiting examples.

Example 1- Isolation and characterisation of expression of DZ2

Plant Material

Seeds of *B. napus* cv Rafal were grown as described by Meakin and Roberts, (1990a) with the following modifications. Single seedlings were potted into 10cm pots, and after vernalization, were re-potted into 21cm pots. At anthesis tags were applied daily to record flower opening. This procedure facilitated accurate age determination of each pod. Pods were harvested at various days after anthesis (DAA). The dehiscence zone was excised from the non-zone material and seed using a scalpel blade (Meakin and Roberts (1990b)) and immediately frozen in liquid N_2 and stored at -70°C.

RNA Isolation

All chemicals were molecular biology grade and bought from either Sigma Chemical Ltd (Dorset, UK), or Fisons (Loughborough, UK). Total RNA was extracted using the polysomal extraction method of Christoffersen and Laties, (1982), with the following alterations. The plant material was ground to a powder in liquid N_2 and then in 10 volumes of extraction buffer (200mM Tris-acetate [pH 8.2], 200mM magnesium

acetate, 20mM potassium acetate, 20mM EDTA, 5% w/v sucrose, after sterilisation 2-mercaptoethanol was added to 15mM and cycloheximide added to a final concentration of 0.1 mg ml^{-1}). The supernatant was then layered over 8 ml 1M sucrose made with extraction buffer and centrifuged in a KONTRON™ (Switzerland) TFT 70.38 rotor at 45,000rpm (150,000g) for 2 hr at 2°C in a Kontron CENTRIKON™ T-1065 ultra-centrifuge. Pellets were then resuspended in 500μl 0.1M sodium acetate, 0.1% SDS, pH 6.0 and phenol/chloroform (1:1 v/v) extracted and the total RNA precipitated. Poly(A)$^+$ RNA was isolated from total RNA extracted, from both the zone and non-zone tissue of 40, 45 and 50 DAA pods, using a Poly(A) QUIK™ mRNA purification kit (Stratagene, Cambridge, UK) following the manufacturers instructions, and then bulked together. Total RNA was also extracted from leaves, stems, seeds and pods using a method described by Dean *et al*, (1985) for use in Northern analyses.

Differential display

This was performed essentially as described by Liang and Pardee (1992) using RNA extracted from 40 DAA pod dehiscence zones and non-zones. First strand cDNA copies of the RNAs (40 DAA DZ/NZ) were made using 50U M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase (50U/μL) (Stratagene) in a 20μL reaction containing 1x M-MLV buffer, 2.5mM dNTPs (Pharmacia), 1μg RNA, 30U RNAse inhibitor (Promega) and 10μM oligo dT anchor primer 7 (5'-TTTTTTTTTTTTGG-3'). The reaction conditions were as follows: 65°C for 5 minutes, 37°C for 90 minutes and 95°C for 5 minutes. Following first strand cDNA synthesis, 60μL dH2O were added and the samples were either used directly for PCR or stored at -20°C.

For PCR, 2μL cDNA were used as template in a 20μL reaction containing 1x PCR buffer, 1mM $MgCl_2$, 2μM dNTPs, 10μM oligo dT anchor primer 7 (5'-

TTTTTTTTTTTTGG-3'), 2.5μM arbitrary primer A (5'- AGC CAG CGA A -3'), 0.5μL 35S-dATP (>1000 Ci/mmol) (Amersham) and 1U *Taq* DNA polymerase (5U/μL) (Gibco BRL). The thermocycling conditions were as follows: 40 cycles of 94^{0}C for 30 seconds, 40^{0}C for 2 minutes, 72^{0}C for 30 seconds followed by 72^{0}C for 5 minutes. The PCR products were fractionated on a 5% polyacrylamide/7M urea gel after addition of 5μL loading buffer (95% (v/v) formamide, 20mM EDTA, 0.05% (w/v) xylene cyanol, 0.05% (w/v) bromophenol blue) to each sample. Following electrophoresis the gel was dried at 80^{0}C under vacuum for 1 hour then exposed to X-ray film (BioMax-MR, Kodak) in a light tight cassette for 48 hours. The dried gel and autoradiogram were aligned so that bands that appeared in the DZ and not in NZ could be cut out and the DNA eluted according to Liang et al. (1995). The eluted PCR products (4μL) were reamplified in a 40μL reaction containing 1x PCR buffer, 1mM $MgCl_2$, 20μM dNTPs, 10μM oligo dT anchor primer 7 (5'-TTTTTTTTTTTTGG-3'), 2.5μM arbitrary primer A (5'- AGC CAG CGA A -3') and 2U *Taq* DNA polymerase (5U/μL) (Gibco BRL) using the following thermocycling conditions: 40 cycles of 94^{0}C for 30 seconds, 40^{0}C for 2 minutes, 72^{0}C for 30 seconds followed by 72^{0}C for 5 minutes. The resulting PCR product was cloned into the TA cloning vector (Invitrogen) and sequenced (Figure 1). In order to prepare an antisense strand-specific riboprobe, the PCR product was subcloned into pBluescript (Stratagene).

Expression analysis and characterisation of DZ2

Northern analysis using an antisense strand-specific riboprobe to the DZ2 PCR product, showed that DZ2 hybridised to a transcript of 0.6kb which is expressed in the DZ of 20-50 DAA pods with a peak in expression at 40DAA. Minimal expression was observed in the pod NZ [Figure 2]. A random-primed labelled DNA probe (Stratagene) of the 330bp DZ2 PCR product (amplified using primers DZ2FL

and DZ2RL - see Figure 1) was used to screen a *B. napus* DZ cDNA library from which, following three rounds of screening to obtain pure plaques, a full length DZ2 cDNA (606bp) was obtained (Figure 1). An antisense strand-specific riboprobe of the full length DZ2 cDNA was hybridised to total RNA extracted from pod DZ/NZ (as in Figure 2), leaf abscission zones (AZ) and non-zones (NZ) (following exposure to 10μL/L ethylene for 72 hours), seed, root, flower and leaf. Figure 3 shows that DZ2 hybridises to a 0.6kb message which is present in the pod DZ at 20-50 DAA with maximum expression at 40DAA. Again there is minimal expression in pod NZ and no apparent expression of DZ2 in AZ, NZ, leaf, root, seed or flower RNA. By the sensitive technique of RT-PCR analysis DZ2 expression can also be detected in anthers and the funiculus, both tissues that contain dehiscent zones

The 606bp cDNA (DZ2) encodes a putative protein of 136 amino acids. Comparison of the DZ2 translated sequence to the OWL protein database [Bleasby and Attwood (1994)] showed low but consistent homology to a group of bacterial proteins comprising two-component regulatory systems. In particular, DZ2 possesses the conserved amino acid residues required for phosphorylation of the receiver domain of the response regulator component (see Figure 4). DZ2 plays a role in a signal transduction cascade resulting at least in one respect in pod shatter. It is therefore a good candidate for down-regulation of pod shatter processes using antisense technology. DZ2 is a novel plant protein in that independent proteins with homology to bacterial receivers are yet to be reported in plants.

The full length cDNA was excised from the pBluescript cloning vector by digestion with *Eco*RI and *Xho*I restriction enzymes (Gibco BRL). Following purification from a 1% agarose gel the 606bp cDNA was random primed labelled (Stratagene) and used to screen a *B. napus* genomic library in the BlueStar vector. Following three rounds of screening to obtain pure plaques, a single genomic clone was isolated

which carries a 15kb genomic DNA insert. The promoter of the DZ2 gene is isolated from this genomic clone using standard techniques (see Example 2).

Example 2 - Isolation and characterisation of the B.napus DZ2B promoter.

To obtain the B.napus DZ2 promoter a B.napus genomic library was screened with a labelled DZ2 probe. The full length cDNA was excised from the pBluescript cloning vector (Stratagene) by digestion with *Eco*RI and *Xho*I restriction enzymes (Gibco BRL). Following purification from a 1% agarose gel the 606bp cDNA was random primed labelled (Stratagene) and used to screen a *B. napus* genomic library in the BlueStar vector (Novagen). Following three rounds of screening to obtain pure plaques, a single genomic clone was isolated which carries a 15kb genomic DNA insert. The region hybridising to DZ2 was sequenced and found to encode a protein homologous to, but not identical to DZ2. This DZ2-like gene was designated DZ2B (Figure 5). The primers DZ2BFL (Figure 5) and T7 were used to PCR out a DZ2B cDNA from the B.napus DZ cDNA library.

5' AACCAAGTCAGTAGAAGTG 3' DZ2BFL
5' AATACGACTCACTATAGG 3' T7

The DZ2 and DZ2B cDNAs are 80% identical (according to the default parameters of the GAP computer program, version 6, Deveraux *et al.*, 1984, and available from the University of Winsconsin Genetics Computer Group (UWGCG)) at the nucleotide level in the region of overlap of the coding sequences (Figure 6a) and the putative proteins encoded by DZ2 and DZ2B are 80% identical (according to the default parameters of the GAP computer program, version 6, Deveraux *et al.*, 1984, and available from the University of Winsconsin Genetics Computer Group (UWGCG)) (Figure 6b). Sequence analysis of more DZ2 and DZ2-like cDNAs and Southern analysis shows that there are two DZ2 genes in B.napus, DZ2 and DZ2B,

each represented by 2 slightly different cDNAs. This is consistent with one gene being encoded by the B.campestris derived-genome and the other from the genome derived from B.oleracea.

RT-PCR with primers specific to DZ2B showed that DZ2B is only expressed in pods. This was confirmed by northern analysis which showed preferential expression in the DZ (Figure 7). Thus DZ2B has a similar pattern of expression as DZ2 and is thus a suitable source of a DZ-expressed promoter.

Primers DZ2BGenF and DZ2BGenR were used to PCR a 1253bp DZ2B promoter fragment (Figure 5).

5' GGCTCTAGACGAACTGCGGAGCAAGG 3' DZ2BGENF
5' CTGCCATGGTCGGTTTTTTTTCTTCGAAC 3' DZ2BGENR

These primers introduced an XbaI site at the 5' end of the PCR fragment and an NcoI site around the initiating Met of DZ2B. Thus the PCR fragment was cloned as an XbaI, NcoI fragment between the XbaI and NcoI sites of pWP272 (WO 99/10389) forming pDZ2B-GUS. The chimeric pDZ2B-GUS-CaMV polyA gene was then transferred as an XbaI, XhoI fragment between the XbaI and SalI sites of pSCV nos-nptII (WO 95/20668) forming pDZ2B-GUS-SCV (Figure 8). The pDZ2B-GUS-SCV binary vector was transferred to the agrobacterial strain pGV2260 and transformed B.napus plants produced by agrobacterial transformation essentially as described in Moloney M et al., (1989). Gus expression is observed in the pod DZ.

Example 3 - Isolation and characterisation of a DZ2 Arabidopsis thaliana homologue

To demonstrate that a DZ2 orthologous gene can be isolated from another plant species the functional equivalent of B.napus DZ2 / DZ2B was isolated from Arabidopsis thaliana. The B.napus DZ2 cDNA was used as a probe to screen an Arabidopsis cDNA library (J. Giraudat, ISV-CNRS, France). Figure 9 shows the sequence of a cDNA (DZ2AT3) that hybridised to the DZ2 probe. DZ2AT3 has 85% nucleic acid identity to DZ2 and 85% to DZ2B (according to the default parameters of the GAP computer program, version 6, Deveraux *et al.*, 1984, and available from the University of Winsconsin Genetics Computer Group (UWGCG)) in the coding regions which are common to all three sequences. The putative peptide encoded by DZ2AT3 has 80% identity to DZ2 and 80% to DZ2B (according to the default parameters of the GAP computer program, version 6, Deveraux *et al.*, 1984, and available from the University of Winsconsin Genetics Computer Group (UWGCG)) in the regions which are common to all three sequences (Figure 10). RT-PCR analysis of RNA isolated from leaves, roots, flowers and siliques showed that DZ2AT3 was specifically expressed in siliques. Southern hybridisation analysis showed that the DZ2AT3, DZ2 and DZ2B probes each identify a single identical band in A.thaliana. This indicates that A.thaliana contains one DZ2 gene in contrast to B.napus which contains two.

The Genome walker kit (Clonetech) was used to isolate the DZ2AT3 promoter from A.thaliana genomic DNA. Nested PCR was performed using primer GW1 first, then AT3GW2 each in conjunction with the Genome Walker kit primer (Figure 9). Figure 11 shows the sequence of the promoter region of DZ2AT3 thus obtained. The primers ATDZ2F and ATDZ2R were used to PCR a 1195bp promoter fragment from the DZ2AT3 genomic sequence (Figure 11).

5' CACTAGTAGGGCACGCGTGGTCG 3' ATDZ2F

5' TCCATGGTCGATTTCTTTTCTCTCAAG 3' ATDZ2R

These primers introduced an SpeI site at the 5' end of the PCR fragment and an NcoI site around the initiating Met of DZ2AT3. Thus the PCR fragment was cloned as an SpeI, NcoI fragment between the XbaI and NcoI sites of pWP272 (WO 99/13089) forming pDZ2AT3-GUS. The chimeric pDZ2AT3-GUS-CaMV polyA gene was then transferred as a SalI, XhoI fragment into the SalI site of pSCV nos-nptII (WO 95/20668) forming pDZ2AT3-GUS-SCV (Figure 12). The pDZ2AT3-GUS-SCV binary vector was transferred to the agrobacterial strain pGV2260 and transformed B.napus plants produced by agrobacterial transformation essentially as described in Moloney M et al., (1989). Gus expression is observed in the pod DZ.

Example 4 - Production of shatter-resistant *B.napus* plants by antisense downregulation of DZ2

Downregulation of the DZ2 gene or reduction in DZ2 protein levels in the pod DZ will result in plants that are resistant (or more resistant than without this modification) to pod shatter. Standard techniques, commonplace in the art, such as the expression of antisense DZ2 mRNA, full sense mRNA, partial sense mRNA or a ribozyme directed against DZ2 mRNA are effective. Expression of these RNAs requires a promoter that is active in the pod DZ at the time at which DZ2 is expressed. Ideally the promoter will be pod DZ-specific, however a useful promoter may be pod-specific or even constitutively active. A suitable promoter would be that of DZ2. Although DZ2 is expressed in the anther DZ, pod DZ and funiculus DZ, DZ2 promoter -GUS fusion studies show that in different transformants the relative level of expression in these three sites is variable but is stability hereditable. Thus some transformants are obtained in which expression is largely or exclusively

confined to the pod DZ. This suggests that the pDZ2 promoter is comprised of distinct elements each specifying expression in a particular DZ. Alternatively the site of transgene integration may influence relative expression levels in the DZ tissues. The DZ2 promoter is therefore linked to the DZ2 cDNA such that the DZ2 is in the antisense orientation forming pDZ2-antiDZ2. This chimeric gene is transferred to the binary vector pNos-NptII-SCV (W0 96/30529). This binary vector is transferred to the agrobacterial strain pGV2260 and transformed *B.napus* plants produced by agrobacterial transformation essentially as described in Moloney M et al., (1989) Plant Cell Reports 8, 238-242. A proportion of transformed *B.napus* plants exhibit reduced levels of DZ2 message and are resistant to pod shatter.

Example 5 - Production of shatter-resistant B.napus plants by antisense downregulation of DZ2

Downregulation of the DZ2 gene or reduction in DZ2 protein levels in the pod DZ will result in plants that are resistant to pod shatter. Techniques such as the expression of antisense DZ2 mRNA, full sense mRNA, partial sense mRNA or a ribozyme directed against DZ2 mRNA will be effective. Expression of these RNAs requires a promoter that is active in the pod DZ at the time at which DZ2 is expressed. Ideally the promoter will be pod DZ-specific, however a useful promoter may be pod-specific or even constitutively active. Although DZ2 / DZ2B is expressed in the anther DZ, pod DZ and funiculus DZ, DZ2B promoter -GUS fusion studies show that in different transformants the relative level of expression in these three sites is variable but is stably hereditable. Thus some transformants are obtained in which expression is largely or exclusively confined to the pod DZ. This suggests that the pDZ2 promoter is comprised of distinct elements each specifying expression in a particular DZ. Alternatively the site of transgene integration may influence relative expression levels in the DZ tissues. Thus a suitable DZ-specific promoter would be that of DZ2, DZ2B, DZ2AT3 or ESJ2A (WO 99/13089).

The primers DZ2FLA and DZ2RLA were used to PCR a 349bp fragment from the DZ2 cDNA:-

5' GGCGAATTCCGGTGAGGAGGCAGTAATC 3' DZ2FLA
5' GGCCCATGGCATACATACACACTTAGAC 3' DZ2RLA

The primers introduce an EcoRI and NcoI site at the ends of the DZ2 PCR fragment. To link the DZ2 PCR fragment in an antisense orientation to the promoter of ESJ2A (pPGL) the DZ2 PCR fragment was cloned as a NcoI, EcoRI fragment between the NcoI and EcoRI sites of pWP272 (WO 99/13089) forming pPGL-DZ2as. The pPGL-antisense DZ2 chimeric gene was transferred as a XbaI, XhoI fragment from pDZ2as into the XbaI and SalI sites of the binary vector pSCV nos-nptII (WO 95/20668) forming pPGL-DZ2as-SCV (Figure 13a).

The pPGL-DZ2as-SCV binary vector was transferred to the agrobacterial strain pGV2260 and transformed B.napus plants produced by agrobacterial transformation essentially as described in Moloney M et al., (1989). A proportion of transformed B.napus plants exhibit reduced levels of DZ2 and DZ2B message and were resistant to pod shatter

Similarly, to link the DZ2 PCR fragment in an antisense orientation to the promoter of DZ2B, the DZ2 PCR fragment is cloned as a NcoI, EcoRI fragment between the NcoI and EcoRI sites of pDZ2B-GUS forming pDZ2B-DZ2as. The pDZ2B-DZ2as chimeric gene is transferred as a XbaI, XhoI fragment from pDZ2B-DZ2as into the XbaI and SalI sites of the binary vector pSCV nos-nptII (WO 95/20668) forming pDZ2B-DZ2as-SCV (Figure 13b).

The pDZ2B-DZ2as-SCV binary vector is transferred to the agrobacterial strain pGV2260 and transformed B.napus plants. Again a proportion of transformed B.napus plants exhibit reduced levels of DZ2 and DZ2B message and are resistant to pod shatter.

Similarly a proportion of B.napus plants transformed with a pDZ23A-DZ2as-SCV construct exhibit reduced levels of DZ2 and DZ2B message and are resistant to pod shatter.

Example 6 - Production of shatter-resistant B.napus plants by antisense downregulation of multiple DZ-expressed genes

In addition to DZ2 several other DZ-expressed genes have been previously isolated and individually downregulated to result in B.napus plants that have increased resistance to pod shatter; namely Sac66 (WO 96/30529 Figure 15), DZ15 (Figure 16) and OSR 7(9) (Figure 17). It is anticipated that downregulation of more than one gene involved in pod shatter will further increase resistance to pod shatter. This could be achieved by combining different transgenes by transformation with several transgenes each designed to downregulate a different DZ-expressed gene or by crossing together B.napus lines that individually are transformed with such transgenes. Such methods are complex either involving transformation with a construct containing multiple chimeric genes or require the maintenance of several transgenic loci in the breeding program. A preferred method is to transform with a chimeric gene consisting of a single promoter driving expression of an antisense or partial sense transcript which is comprised of elements of all the DZ-expressed genes to be downregulated. Similarly a single promoter could be used to drive the expression of multiple ribozymes each targeted against a different DZ-expressed gene. The use of a single promoter to drive expression of a combination of

antisense, partial sense and ribozymes is also possible. Ideally the promoter will be pod DZ-specific, however a useful promoter may be pod-specific or even constitutively active. A suitable DZ-specific promoter would be that of DZ2, DZ2B, DZ2AT3 or ESJ2A.

Consequently the ESJ2A promoter was linked to a multiple antisense gene consisting of elements of Sac66, DZ2, DZ15 and OSR 7(9) in the following manner:- The original DZ15 PCR product in pCRII (Invitrogen) (see Figure 16) was cloned as an EcoRI fragment into pBluescript SK (Stratagene) forming pDZ15-BS, such that the DZ15 3' end is nearest the SstI site of the vector . T7 and DZ15RL primers were used to PCR a 456bp DZ15 fragment from pDZ15-BS which was cloned into the EcoRV site of pGEM5zf (Promega) forming pWP351, such that the DZ15 3' end is nearest the SphI site of the vector.

5' AATACGACTCACTATAGG 3' T7
5' AACAGCTGAAAACCTCACGAAG 3' DZ15RL

The EcoRI, NcoI fragment of pWP351 cloned between the EcoRI and NcoI sites of pDZ2-BS forming pWP356. pDZ2-BS consists of the DZ2 cDNA cloned as an EcoRI, XhoI fragment into pBluescript SK such that the 3' end is nearest the KpnI site of the vector. A 361bp Sac66 fragment was PCRed from the Sac66 cDNA (WO 96/30529) using the primers F1 and RI which introduce NcoI and PstI sites into the ends of the PCR product.

5' GGCCCATGGCTGCCAAGCTTTGAGTAGC 3' F1
5' GGCCTGCAGTGCCTAGGATCTGGAAGC 3' RI

The Sac66 PCR product was cloned as an NcoI. EcoRI fragment between the NcoI and EcoRI sites of pWP272 (WO 99/13089) forming pWP288A. EcoRI DZ15+DZ2 and OSR 7(9) fragments from pWP356 and pOSR 7(9)-CRII were cloned into the EcoRI site of pWP288A such that DZ15+DZ2 and OSR 7(9) are in an antisense orientation with respect to PGL promoter. (pOSR 7(9)-CRII consists of the 306bp OSR 7(9) PCR fragment (see Figure 17) cloned into pCRII (Invitrogen)). The chimeric pPGL-antisense Sac66+DZ2+Dz15+OSR 7(9) gene was transferred as a XbaI. XhoI fragment into the XbaI and SalI sites of the binary vector pSCV nos-nptII (WO 95/20668) forming pWP357-SCV (Figure 14). The pWP357-SCV binary vector was transferred to the agrobacterial strain pGV2260 and transformed B.napus plants produced by agrobacterial transformation essentially as described in Moloney M et al., (1989) Plant Cell Reports 8, 238-242.

Resistance to podshatter was measured using an impact pendulum device (Liu X-Y, Macmillan RH and Burrow RP 1994 Journal of Texture Studies 25 p179-189) (Table1). The mean energy values shown in Table 1 represent the energy required to rupture the pod on impact with the pendulum. These values are an average from measurements of 20 mature pods. The letters A to L indicate grouping of transformants with significantly different podshatter resistance (ie Group A is significantly different from B when analysed by ANOVA using a Fisher PLSD analysis with a significance level of 95% (Statview 512+). Lines with a number of letters are not significantly different from other lines sharing the same letter. The results shown in Table 1 indicate that 24 lines exhibited significantly higher resistance to podshatter than non-transformed controls whilst 17 lines were not significantly different from the control. The degree and frequency of pod shatter resistance achieved with pWP357-SCV was greater than that obtained by transformation with constructs that downregulate a single DZ-expressed gene.

Table 1

PLANT ID	MEAN ENERGY												
A213-24	6.752	A											
A213-53	5.203		B										
A213-34	3.864			C									
A213-21	3.673			C									
A213-4	3.54			C									
A213-61	3.516			C									
A214-30	3.46			C									
A214-27	3.397			C									
A213-8	3.277			C									
A213-70	3.271			C	D								
A214-13	3.182			C	D	E							
A213-11	3.182			C	D	E	F						
A213-69	3.005				D	E	F	G					
A213-60	2.945				D	E	F	G	H	.			
A214-7	2.843				D	E	F	G	H	I			
A213-64	2.687				D	E	F	G	H	I	J		
A214-14	2.613				D	E	F	G	H	I	J		
A213-28	2.581				D	E	F	G	H	I	J		
A213-9	2.547				D	E	F	G	H	I	J		
A213-42	2.442					E	F	G	H	I	J		
A213-31	2.431						F	G	H	I	J		
A214-10	2.42							G	H	I	J		
A213-38	2.295								H	I	J		
A214-8	2.26								H	I	J		
A213-19	2.213								H	I	J		
A214-25	2.128								H	I	J	K	
A213-38	2.059									I	J	K	
A213-16	2.005									I	J	K	
A213-63	1.928									I	J	K	
A213-33	1.91										J	K	L
A213-32	1.901										J	K	L
A213-37	1.791										J	K	L
RV27CONT	1.787											K	L
A213-10	1.623											K	L
A213-58	1.595												L
A214-24	1.55												L
A213-3	1.518												L
A213-27	1.495												L
A213-40	1.395												L
A213-47	1.315												L
A213-43	1.286												L
A213-30	1.241												L
													L

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CLAIMS

1. Nucleic acid encoding a signal transduction protein involved in the process of dehiscence.

2. Nucleic acid as claimed in claim 1 wherein the process involves the production of a hydrolytic enzyme.

3. Nucleic acid as claimed in claim 1 or claim 2 which is naturally expressed in a dehiscence zone.

4. Nucleic acid encoding a protein wherein the protein:

   a) comprises the amino acid sequence shown in Figure 1 or;

   b) has one or more amino acid deletions, insertions or substitutions relative to a protein as defined in a) above and has at least 40% amino acid sequence identity therewith; or

   c) is a fragment of a protein as defined in a) or b) above, which is at least 10 amino acids long.

5. Nucleic acid as claimed in any one of claims 1 to 4 which comprises the sequence set out in Figure 1 or a fragment thereof which is at least 30 bases long.

6. Nucleic acid, as claimed in any one of claims 1 to 5 in combination with one or more further nucleic acid sequence which is dehiscence-zone expressed.

7. Nucleic acid which is antisense to nucleic acid as claimed in any one of claims 1 to 6.

8. Nucleic acid as claimed in any one of claims 1 to 7 including a promoter or other regulatory sequence which controls expression of the nucleic acid.

9. Nucleic acid which is the naturally occurring promoter or other regulatory sequence which controls expression of nucleic acid as claimed in any one of claims 1 to 8.

10. Nucleic acid as claimed in any one of claims 1 to 9 which is in the form of a vector.

11. A cell comprising nucleic acid as claimed in any one of claims 1 to 10.

12. A plant cell as claimed in claim 11.

13. A process for obtaining a cell as claimed in claim 11 or claim 12 comprising introducing nucleic acid as claimed in any one of claims 1 to 10 into said cell.

14. A plant or a part thereof comprising a cell as claimed in claim 11 or claim 12.

15. Propagating material or a seed comprising a cell as claimed in claim 11 or claim 12.

16. A process for obtaining a plant or plant part as claimed in claim 14 or claim 15 comprising obtaining a cell as claimed in claim 11 and growth thereof or obtaining a plant, plant part, or propagating material as claimed in claim 14 or claim 15 and growth thereof.

17. A signal transduction protein involved in the process of plant dehiscence.

18. A protein which:

a) comprises the amino acid sequence shown in Figure 1 or;

b) has one or more amino acid deletions, insertions or substitutions relative to a protein as defined in a) above, and has at least 40% amino acid sequence identity therewith; or

c) a fragment of a protein as defined in a) or b) above which is at least 10 amino acids long.

19. A protein as claimed in claim 17 or claim 18 which is isolated or recombinant.

20. A process for regulating/controlling dehiscence in a plant or a part thereof, the process comprising obtaining a plant or part thereof as claimed in claim 14.

21. A process as claimed in claim 20 which comprises obtaining a plant cell as claimed in claim 21 or part of a plant as claimed in claim 14 and deriving a plant therefrom.

22. A process as claimed in claim 20 which comprises obtaining propagating material or a seed as claimed in claim 15 and deriving a plant therefrom

23. A process as claimed in claim 20 wherein the dehiscence is of a pod or of an anther.

24. Use of nucleic acid as claimed in any one of claims 1 to 10 in the regulation/control of plant dehiscence.

25. Use of nucleic acid as claimed in any one of claims 1 to 10 as a probe.

26. Use of nucleic acid as claimed in any one of claims 1 to 10 in the production of a cell, tissue, plant part thereof or propagating material.

27. Nucleic acid comprising one or more of the underlined sequences as set out in Figure 1, or one or more of the primer sequences in Figure 5, 9 and/or 11.

28. Use of the nucleic acid as claimed in claim 27 as a PCR primer.

29. Use of a protein as claimed in any one of claims 17 to 19 as a probe.

FIG. 1

NUCLEIC ACID AND PREDICTED PROTEIN SEQUENCE OF DZ2

```
                                             NcoI
1   GGCACGAGCAGAATCGAAGATGGCAACAAAATCCATGGGAGATATCGAGAAAATAAAGAA 60
                         M  A  T  K  S  M  G  D  I  E  K  I  K  K

61  GAAACTAAACGTGTTGATCGTCGATGATGATCCACTAAACCTTATAATTCATGAGAAGAT 120
     K  L  N  V  L  I  V  D  D  D  P  L  N  L  I  I  H  E  K  I
                                ↓                      DZ2FL→
121 CATCAAAGCGATTGGGGGTATTTCACAGACAGCGAATAACGGTGAGGAGGCAGTAATCAT 180
     I  K  A  I  G  G  I  S  Q  T  A  N  N  G  E  E  A  V  I  I

181 CCACCGTGACGGCGGCTCATCTTTTGACCTTATCCTAATGGATAAAGAAATGCCCGAGAG 240
     H  R  D  G  G  S  S  F  D  L  I  L  M  D  K  E  M  P  E  R

241 GGATGGTGTTTCGACAACTAAGAAGCTAAGAGAAATGGAAGTGAAGTCAATGATTGTTGG 300
     D  G  V  S  T  T  K  K  L  R  E  M  E  V  K  S  M  I  V  G

301 GGTGACTTCACTGGCTGACAATGAAGAGGAGCGCAGGGCTTTCATGGAAGCTGGACTTAA 360
     V  T  S  L  A  D  N  E  E  E  R  R  A  F  M  E  A  G  L  N
```

```
361 CCATTGCTTGGCAAAACCGTTAACCAAGGACAAGATCATCCCTCTCATTAACCAACTCAT 420
     H  C  L  A  K  P  L  T  K  D  K  I  I  P  L  I  N  Q  L  M
                                                        ← DZ2RL
421 GGATGCTTGATGGATATATATTTTATATTATGGAAACACACATAATAACGTCTAAGTGTG 480
     D  A  *

481 TATGTATGCATAGATACTTGCATGTGTGTGTTTTAGAATTTAGGGTTCTTTATCGTCCGT 540
                                HindIII
541 GATATATAATCATGTAAGTTGTTGCTTTAAGCTTATAAAATATTTAAATAAGGGTTTCCT 600

601 CTACC
```

The primer sites for **DZ2FL** and **DZ2RL** are underlined, as are the recognition sequences for *Nco*I and *Hin*dIII restriction enzymes. Shown in bold are the conserved amino acid residues required for phosphorylation. The extent of the original PCR product isolated by differential display is shown by ↓.

FIG. 1 CONT'D

Alignment of the predicted protein sequence of DZ2 with those of bacterial response regulator proteins.

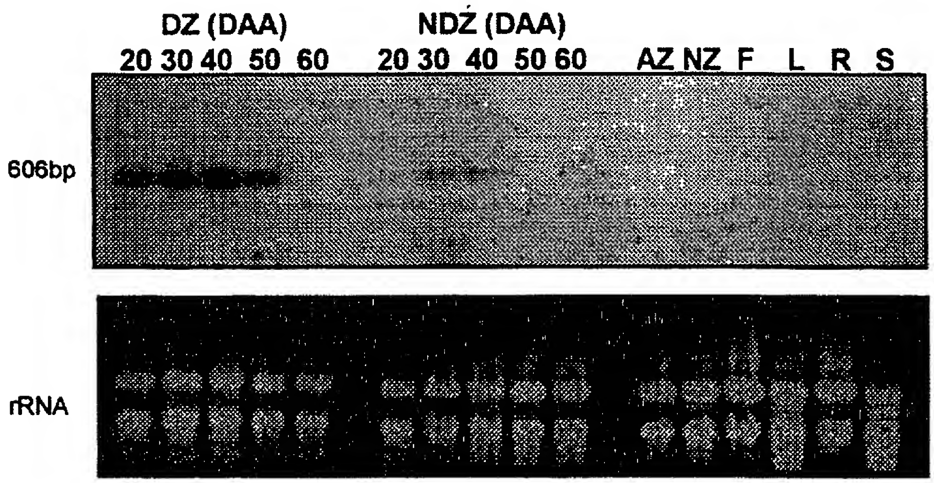
```
        1                                                      50
DZ2     MATKSMGDIE KIKKKLNVLI VDDDPLNLII HEKIIKAIG. GISQTANNGE
OMPR    .......... .MQENYKILV VDDDMRLRAL LERYLTEQGF .QVRSVANAE
PHOB    .......... ...MARRILV VEDEAPIREM VCFVLEQNGF .QPVEAEDYD
NTRC    .......... ..MQRGIVWV VDDDSSIRWV LERALAGAGL .TCTTFENGN
SPOOF   .......... ..MMNEKILI VDDQYGIRIL LNEVFNKEGY .QTFQAANGL
CHEY    .......... MADKELKFLV VDDFSTMRRI VRNLLKELGF NNVEEAEDGV
ETR     .......... .....LKVLV MDENGVSRMV TKGLLVHLGC EVTTVSSNEE

        51                                                    100
DZ2     EAVIIHRDGG SSFDLILMDK EMPERDGVST TKKLREMEVK SM..IVGVTS
OMPR    QMDRLLTR.. ESFHLMVLDL MLPGEDGLSI CRRLRSQS.. NPMPIIMVTA
PHOB    SAVNQLNE.. PWPDLILLDW MLPGGSGIQF IKHLKRESMT RDIPVVMLTA
NTRC    EVLAALAS.. KTPDVLLSDI RMPGMDGLAL LKQIKQ..RH PMLPVIIMTA
SPOOF   QALDIVTK.. ERPDLVLLDM KIPGMDGIEI LKRMKV..ID ENIRVIIMTA
CHEY    DALNKLQA.. GGYGFVISDW NMPNMDGLEL LKTIRADGAM SALPVLMVTA
ETR     ....CLRVVS HEHKVVFMDV CMPGVENYQI ALRI...... .HXPLLVALS

        101                                                   150
DZ2     LADNEEERRA FMEAGLNHCL AKPLTKDKII PLINQLMDA. ..........
OMPR    KGEEVDRIVG .LEIGADDYI PKPFNPRELL ARIRAVLRRQ ANELPGAPS.
PHOB    RGEEEDRVRG .LETGADDYI TKPFSPKELV ARIKAVMRRI SPM.......
NTRC    HSDLDAAVSA .YQQGAFDYL PKPFDIDEAV ALVERAISHY QEQQQPRNIE
SPOOF   YGELDMIQES .KELGALTHF AKPFDIDEIR DAVKKYLPLK SN........
CHEY    EAKKENIIAA .AQAGASGYV VKPFTPATLE EKLNKIFEKL GM........
ETR     GNTDKSTKEK CMSFGLDGVL LKPVSLDNIR DVLSDLL... ....... ..

      151
DZ2     ....
OMPR    ....
PHOB    ....
NTRC    VNGP
SPOOF   ....
CHEY    ....
ETR     ....
```

OmpR = *E. coli* (Involved in osmoregulation)
PhoB = *E. coli* (Involved in phosphate utilisation)
NtrC = *S. typhimurium* (Involved in nitrogen utilisation)
SpoOF = *B. subtilis* (Involved in sporulation)
CheY = *E. coli* (Involved in chemotaxis)
ETR = *A. thaliana ETR1* gene encoding an ethylene receptor (partial amino acid sequence)

The predicted protein sequence of DZ2 is shown in bold as are the conserved amino acid residues required for phosphorylation of the protein

FIG. 2

Expression analysis of DZ2 in various plant organs using Northerns



DZ = Pod dehiscence zone (20-60 DAA)
NDZ = Pod non-zone (20-60DAA)
AZ = Leaf abscission zone
NZ = Non-zone (stem)
F = Flower
L = Leaf
R = Root
S = Seed

FIG. 3

Comparison of bacterial two-component regulatory systems with DZ2

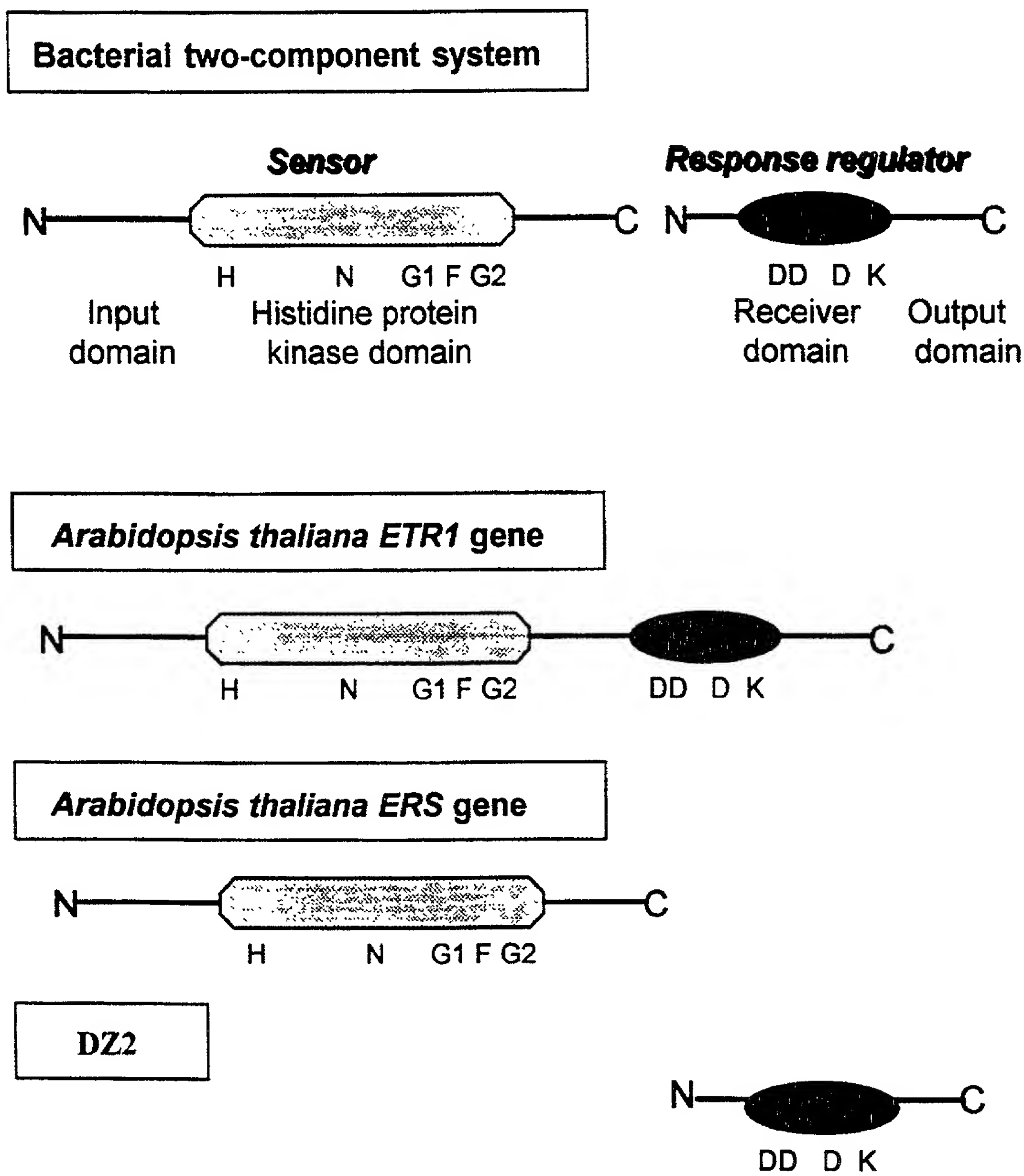


FIG. 4

FIG. 5

```
TATATAAATACGGTTTAACAGATATGTTCTGGTTATAAATGTAATTCNATGTGCCNNTCAANTTTTATTTTNATTNGT   78

TNTACTAGGGACATTAGTTTTAACNTTTTATATATCATGTAACAAAAAAAAAAAAAACNTTTTATATNTCAACTATGA   156

GCAATTATTCTTATAGTGTTTTCTTTTTCCAGAAATTTGACGACAACCTAACTAAAACAATTTAATTTGACGTTAGTT   234

AAGTAATTTATATAGATGGATAAATTGAGCAAGCACATTACGAACTGCGGATCAAGGAGAGTCACAATTTAATTCTTA   312
                                  GGCTCTAGACGAACTGCGGAGCAAGG ->    DZ2BGENF
                                     XbaI

CGTTATACACAAAATTATCTAAATACTATATATATATACAGCTGCATGCTACGATAATGATCAAATGTTTATGTACTT   390

TTCAGCGAAAATTCTTGTCGCCATACATTACTGTGTTAATGAATCATTAAATATGTGAAGGAGGAAAAGAGTACAAAA   468

GGAGTTTTGTTGAGGCATTTCGCAGACACTGAAATGTGAATAATAATAAAGGAATTGCCGAATTGATTTCTAGTTGGT   546

GAAGTGGGTGAAAATTGTATGTCCATTGCTTATAAACTATAAAATATAATATNTTNATATTATCACTNTGGACATTAG   624

TNNGATAGACCCTAGCTAAAATTTTTAAAAATTATACATTCATTTTCTNAAGTACCAAACTTAATTATCACAATCGGA   702

TAAAATTGTTTAAGAAACCATTACAAACTCAGCTTGTGGACTCTGAGAGAAACTAAGAGCTAGACATACGGTTAGTAG   780

TGTAGCCGCATTTTTTATGCTTAATTTGCTTAAGCATGACTTCTATGCTCCTTGATGATATTTATTTTAATATCCTAG   858

GACATATGGATTTGATAAAGATCTTATCAACCTTTCAACAAGACCATTAGCTCAACAAACAAAATACTGAAAGTATAT   936
```

```
AATCTTGGTTACAGAATTCTTATGCCAAAAATATCATAATATATATAGAATTCGGTTATGATTAAGATGAATTATTTA  1013

ATTAATATATTTTTCACTTTTGTTTTCTTATGTATTCTTAGTATTTGTTCACCATATTGACCGATTGGTGTCATATTA  1092

GTTTGGTAAGACAACTCAGTTGCAACGATGCAGATTACATTTCAGGAAGATTCATGTAAGAAAGATATTTCGCTTTGT  1170

GGTGTGAAAATATGCCTCTTTCACTTTTTTTCAACTATAAATTTCGATCGATGTATCTACGTTCTTAACACAATTCAC  1248

AATCTTCTTTAGAATCCAAAATTGTAAGCCGCTTTCTAATCTCTTTCTCAGTATACATATGTAATATGTATGCATATA  1326

TTATTATTCATAATACAAACACGAACCCATGCATGCAAGAAGATAGTTACACGCTCATAACAAACACAAAAAAACATA  1404

CGCATGCATTAGAACACTTGTATGTTAATTTCCATAATGTTTTGCATAAACATTCTTCGTTTTAATTAGCTTCTTTTT  1482

                                 NcoI
      <- GTTCGAAGAAAAAAAAACCGACCATGGCAG   DZ2BGENR                    AACCAAG
GTGTGAAGATTGTTCGAAGAAAAAAAAACCGAAGATGGCAACAACGTCAACATCCACGGGAGATATCAAGAAAACCAAG  1560
                                    M  A  T  T  S  T  S  T  G  D  I  K  K  T  K

TCAGTAGAAGTG ->  DZ2BFL
TCAGTAGAAGTGAAGAAGAAACTTAACGTGTTGATCGTTGATGATGATACAGTAATTCGTAAACTTCACGAGAATATC  1638
S  V  E  V  K  K  K  L  N  V  L  I  V  D  D  D  T  V  I  R  K  L  H  E  N  I

ATCAAATCGATCGGTGGAATTTCACAGACGGCTAAGAACGGTGAGGAGGCAGTGAACATCCACCGCGACGGCAATGCA  1716
I  K  S  I  G  G  I  S  Q  T  A  K  N  G  E  E  A  V  N  I  H  R  D  G  N  A ->
```

FIG. 5 CONT'D

FIG. 6

A) Dz2B cDNA segence (top) -
Dz2 sequence (bottom)

```
TCGTCNAT
GGCACGAGCAGAATCGAAGATGGCAACAAAATCCATGGGAGATATCGAGAAAATAAAGAAGAAACTAAACGTGTTGATCGTC
10 20 30 40 50 60 70 80

10 20 30 40 50 60 70 80
GATGATCCTGTAATACGTAAACTTCACGAGATTATCATCAAATCAATCGGTGGA---ATTTCACAGACAGCTAAGAACGGTG
GATGATGATCCACTAAACCTTATAATTCATGAGAAGATCATCAAAGCGATTGGGGGTATTTCACAGACAGCGAATAACGGTG
90 100 110 120 130 140 150 160

90 100 110 120 130 140 150 160
AGGAGGCAGTGAACATCCACCGCGACGGCAATGCATCTTTCGACCTTATCCTAATGGATAAAGAAATGCCCGAGAGGGATGG
AGGAGGCAGTAATCATCCACCGTGACGGCGGCTCATCTTTTGACCTTATCCTAATGGATAAAGAAATGCCCGAGAGGGATGG
170 180 190 200 210 220 230 240

170 180 190 200 210 220 230 240 250
ACTTTCGGCAACTAAGAAGCTAAGAGAAATGAAAGTGACGTCTATGATTATTGGGGTGACGACACTGGCTGACAATGAAGAG
TGTTTCGACAACTAAGAAGCTAAGAGAAATGGAAGTGAAGTCAATGATTGTTGGGGTGACTTCACTGGCTGACAATGAAGAG
250 260 270 280 290 300 310 320
```

```
        260       270       280       290       300       310       320       330
GAACGTAAGGCTTTCATGGAAGCTGGACTTAACCATTGCTTGGCAAAACCCTTAAGCAAAGCCAAGATCCTCCCTCTCATCA
GAGCGCAGGGCTTTCATGGAAGCTGGACTTAACCATTGCTTGGCAAAACCGTTAACCAAGGACAAGATCATCCCTCTCATTA
 330       340       350       360       370       380       390       400       410

        340       350       360       370       380       390       400       410
ACAATCTCATGGATGCTTGATGGATGGATGAATTGTCGCCACTACATATCTACATTATACAAATATGAAAAACACATATAAT
ACCAACTCATGGATGCTTGATGGATATATATTTTATATTATG-------------------------GAAACACACATAAT
          420       430       440       450                                 460

     420       430       440       450       460       470       480       490
AACGTCATACACCTGTGTG--TGTATGCATAGATATCTATCCGCATGTGTGTTTTTAGGGTTGTTATGTTTGATTTTTATTG
AACGTCTA-----AGTGTGTATGTATGCATAGATACTTGCAT--GTGTGTGTTTTAGAATTTAGGGTTCTTTATCGTCCGTG
   470            480       490       500          510       520       530       540
```

FIG. 6 CONT'D

```
        500       510       520       530       540       550       560       570
TGCGTGGCGTGATATACAATCANGTNAGTCGTTACTTTTGGCTTATAAAATAAT-GAATAAGATTTGTTAAAAATAAAAAAA
ATATATAATCATGTAAGTTGTTGCTTTAA-----------GCTTATAAAATATTTAAATAAGGGTTTCCTCTACC
           550       560       570                580       590       600
```

B) Dz2B peptide seqence (top) -
Dz2 peptide sequence (bottom)

```
                                 10        20        30        40        50        60
                       VXDDPVIRKLHEIIIKSIGGISQTAKNGEEAVNIHRDGNASFDLILMDKEMPERDGLSATKK
MATKSMGDIEKIKKKLNVLIVDDDPLNLIIHEKIIKAIGGISQTANNGEEAVIIHRDGGSSFDLILMDKEMPERDGVSTTKK
         10        20        30        40        50        60        70        80

       70        80        90        100       110
LREMKVTSMIIGVTTLADNEEERKAFMEAGLNHCLAKPLSKAKILPLINNLMDA
LREMEVKSMIVGVTSLADNEEERRAFMEAGLNHCLAKPLTKDKIIPLINQLMDA
       90        100       110       120       130
```

FIG. 6 CONT'D

FIG. 7

NORTHERN ANALYSIS OF EXPRESSION OF
DZ2B IN PODS AND OTHER TISSUES

DZ = POD DEHISCIENCE ZONE (20 - 06 daa)
NZ = POD NON-ZONE
AZ = ABSCISSION ZONE
F = FLOWER
L = LEAF
R = ROOT
S = SEED

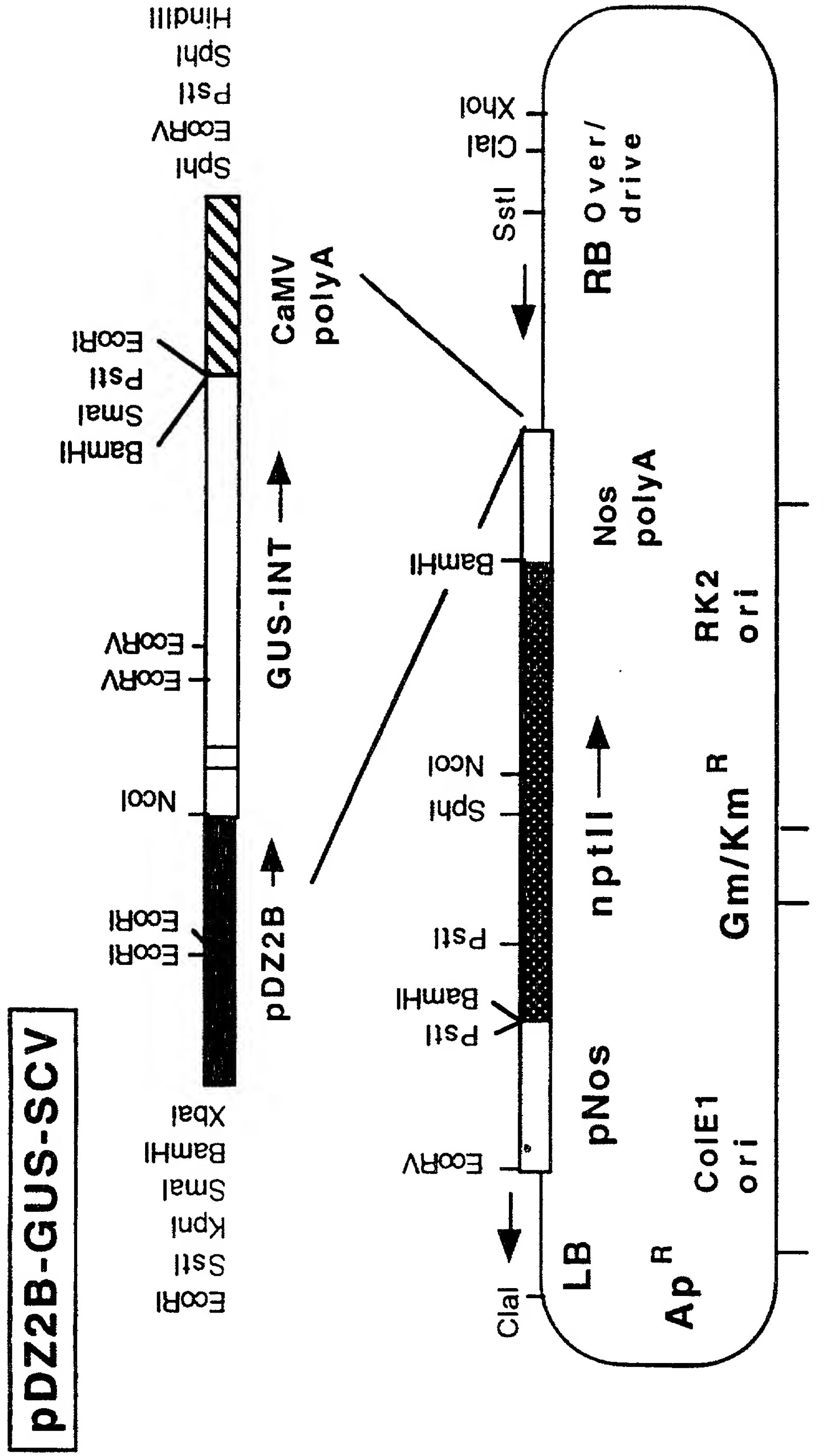

pDZ2B-GUS-SCV
HindIII
SphI
PstI
EcoRV
SphI
EcoRI
PstI
SmaI
BamHI
CaMV polyA
GUS-INT
EcoRV
EcoRV
NcoI
EcoRI
EcoRI
pDZ2B
XbaI
BamHI
SmaI
KpnI
SstI
EcoRI
XhoI
ClaI
SstI
RB Over/drive
Nos polyA
BamHI
RK2 ori
NcoI
SphI
nptII
Gm/Km R
PstI
BamHI
PstI
pNos
EcoRV
ColE1 ori
LB
Ap R
ClaI
SphI
KpnI
SstI
SphI

FIG.8

09/646679

FIG. 9

```
ATATATGTGATACAGATACATCTATATACAAATTAAACACGAAACCATACATGCACGGTGTGATCACACACGCACACA   78

CATAGAAACATAAACACGCAATAATTTCTATACAGTTTAATTTCATTTTTAACTTACTTCTTTTTTTTTGGTGAAGAT  156

TCTTGAGAGAAAAGAAATCGAAGATGGCAACAAAATCCACCGGAGGTACCGAGAAAACCAAGTCGATAGAAGTGAAGA  234
                         M  A  T  K  S  T  G  G  T  E  K  T  K  S  I  E  V  K  K

                                                                          <- CGATCG
AGAAACTAATCAACGTGTTGATCGTCGATGATGATCCATTAAACCGTAGACTCCACGAGATGATCATCAAAACGATCG  312
  K  L  I  N  V  L  I  V  D  D  D  P  L  N  R  R  L  H  E  M  I  I  K  T  I  G

              <- CGAATAACGGTGAGGAGGCATTAATCA  GW1
GAGGAATTTCTCAGACTGCAA  AT3GW2
GAGGAATTTCTCAGACTGCAAAGAATGGCGAAGAGGCNGTGATCCTCCACCGTGACGGCGAAGCATCTTTCGACCTTA  390
  G  I  S  Q  T  A  K  N  G  E  E  X  V  I  L  H  R  D  G  E  A  S  F  D  L  I
```

```
TTCTAATGGATAAGGAAATGCCTGAGAGGGATGGAGTTTCGACAATTAAGANGCTAAGAGAAATGAAAGGGACGTCAA  468
 L  M  D  K  E  M  P  E  R  D  G  V  S  T  I  K  X  L  R  E  M  K  G  T  S  M

TGATCGTTGGGGTAACGTCAGTAGCTGACCAAGAAGAAGAGCGTAAGGCTTTTATGGAAGCTGGGCTCAACCATTGCT  546
 I  V  G  V  T  S  V  A  D  Q  E  E  E  R  K  A  F  M  E  A  G  L  N  H  C  L

TGGAAAAACCCTTAACCAAGGCCAAGATCTTCCCGCTCATTAGCCACCTCTTCGATGCTTGATGGATGAAGGCTCATT  624
 E  K  P  L  T  K  A  K  I  F  P  L  I  S  H  L  F  D  A  •

AATGTATCTATATTTTCAATCATGAAATCACCTACACGTGTATTTGACACAAAAATCTGCATTTGTTGTGATATAGGG  702

TTTCTCATATCTATGTTTGATTTATTTTCTTATCGTCCGAGGTAAAATCATGCAAGTCATTTCTTTTGGCTAATAAAA  780

TATTAAAATAAGGTTTTCTCAAAAAAAAAAAAAAAAAA                                          818
```

FIG. 9 CONT'D

FIG. 10

A) DZ2AT3 peptide sequence (top)
DZ2 peptide sequence (bottom)

```
        10        20        30        40        50        60        70        80
MATKSTGGTEKTKSIEVKKKLINVLIVDDDPLNRRLHEMIIKTIGGISQTAKNGEEXVILHRDGEASFDLILMDKEMPERDG
         MATKSMGDIEKIKKKLNVLIVDDDPLNLIIHEKIIKAIGGISQTANNGEEAVIIHRDGGSSFDLILMDKEMPERDG
                  10        20        30        40        50        60        70

       90       100       110       120       130       140
VSTIKXLREMKGTSMIVGVTSVADQEEERKAFMEAGLNHCLEKPLTKAKIFPLISHLFDA
VSTTKKLREMEVKSMIVGVTSLADNEEERRAFMEAGLNHCLAKPLTKDKIIPLINQLMDA
    80       90       100       110       120       130
```

B) DZ2AT3 peptide sequence (top)
DZ2B peptide sequence (bottom)

```
                10        20        30        40        50        60        70        80
MATKSTGGTEKTKSIEVKKKLINVLIVDDDPLNRRLHEMIIKTIGGISQTAKNGEEXVILHRDGEASFDLILMDKEMPERDG
                          • •••| •|•••|•••|•••••••••••• • |••••|•••••••••••••••••
                          VXDDPVIRKLHEIIIKSIGGISQTAKNGEEAVNIHRDGNASFDLILMDKEMPERDG
                                 10        20        30        40        50

      90        100       110       120       130       140
VSTIKXLREMKGTSMIVGVTSVADQEEERKAFMEAGLNHCLEKPLTKAKIFPLISHLFDA
|•| • ••••• ••••|•••||••|••••••••••••••• •••|••••|•••||• ••
LSATKKLREMKVTSMIIGVTTLADNEEERKAFMEAGLNHCLAKPLSKAKILPLINNLMDA
   60        70        80        90        100       110
```

FIG. 10 CONT'D

FIG. 11

```
GTAATGCGACTCACTATAGGGCACGCGTGGTCGACGGCCCGGGCTGGTCCTCATTCGTATTGGGCCCAATGGGCTACT   78
          CACTAGTAGGGCACGCGTGGTCG -> ATDZ2F
            SpeI

AAAACAGTTTCACGATTGTTTTTTTTTTTTTTTTTTTTTAATTTTAACATGTATGTGGATATTTGGCTATAAATTATG  156

TAAAAAATTTCACGATAGATTGTTGAATTTTTGAATTTCGAGTTAAAATATCTTCAAATTACCTCACATTTACAAAAA  234

GGTAGAACTGTTGAAAAACTAATGCTCTATAAAACACTAGACAATAACAAAATACGTAATGCGTAAAGAACCTAAATT  312

ATGATTTTATTTATCTTTCTTCCTTTTTCCGTGAGTATAAGCCATTTTTCATAGTAAAGCATTACGAATACGACATTG  390

AACACTACTGACATATAAAGTAGTAGATTTTGATGGGTTAACTTGTATGCTTAATTTGCTTAAGCATGAACTTCAATG  468

CTTTTATAAAAGTACTTCATGAGAATATTCCTCGTTCTATACTAGCAGAAGGGTTCGATAGTGATTTTACAACCGTTC  546

AACAAAACCTTTAAACCCAAAAAACCAAAGAATGAAAGTATCTAAACTTGATTATACATTTCTTGTCTAAATTATCAA  624

ATAACATACTCTCTTTTGTTTACTTATAAACGATATGAAAGAAATAAATAAAAAGAACATAGAATCTTATTATGATCT  702
```

```
AGAAGAATTAATTAAAGAAATATATATATATATTTTTTTCATTTCTACTCATGTTTCTTATACATTCTTTAAATTTGTT   780

CACCATTTTGATTTACTTGTTCTCATATTAGTTTGTTATACAACTCACTTAGAATAATGTAGATTACATTTCAGCCAA   858

ATTCATGTAAAAGATGCTTTTCTTTGTGATGTTTTTAAAATGCTTTCTTTTCACTTTTTTTCTTTCTTAACTATAAAT   936

CTTGATCGAATGCCTACCTTCTTAGAACATAAGATCTTCTTTAAAATCCAAAATCGTAGGCCACTATTTCATTATACT   1013

TATGTAATATATGTGATACAGATACATNTATATACAAATTAAACACGAAACCATACATGCACGGTGTGATCACACACG   1092

CACACACATAGAAACATAAACACGCAATAATTTCTATACAGTTTAATTTCATTTTTAACTTACTTCTTTTTTTTTGGT   1170

                             NcoI
     <- CTTGAGAGAAAAGAAATCGACCATGGA
GAAGATTCTTGAGAGAAAAGAAATCGAAGATGGCAACAAAATCCACCGGAGGTACCGAGAAAACCAAGTCGATAGAAG   1248
                              M  A  T  K  S  T  G  G  T  E  K  T  K  S  I  E  V

TGAAGAAGAAACTAATCAACGTGTTGATCGTCGATGATGATCCATTAAACCGTAGACTCCACGAGTGTCATCAAAA   1324
  K  K  K  L  I  N  V  L  I  V  D  D  D  P  L  N  R  R  L  H  E  C  H  Q  ->
```

FIG. 11 CONT'D

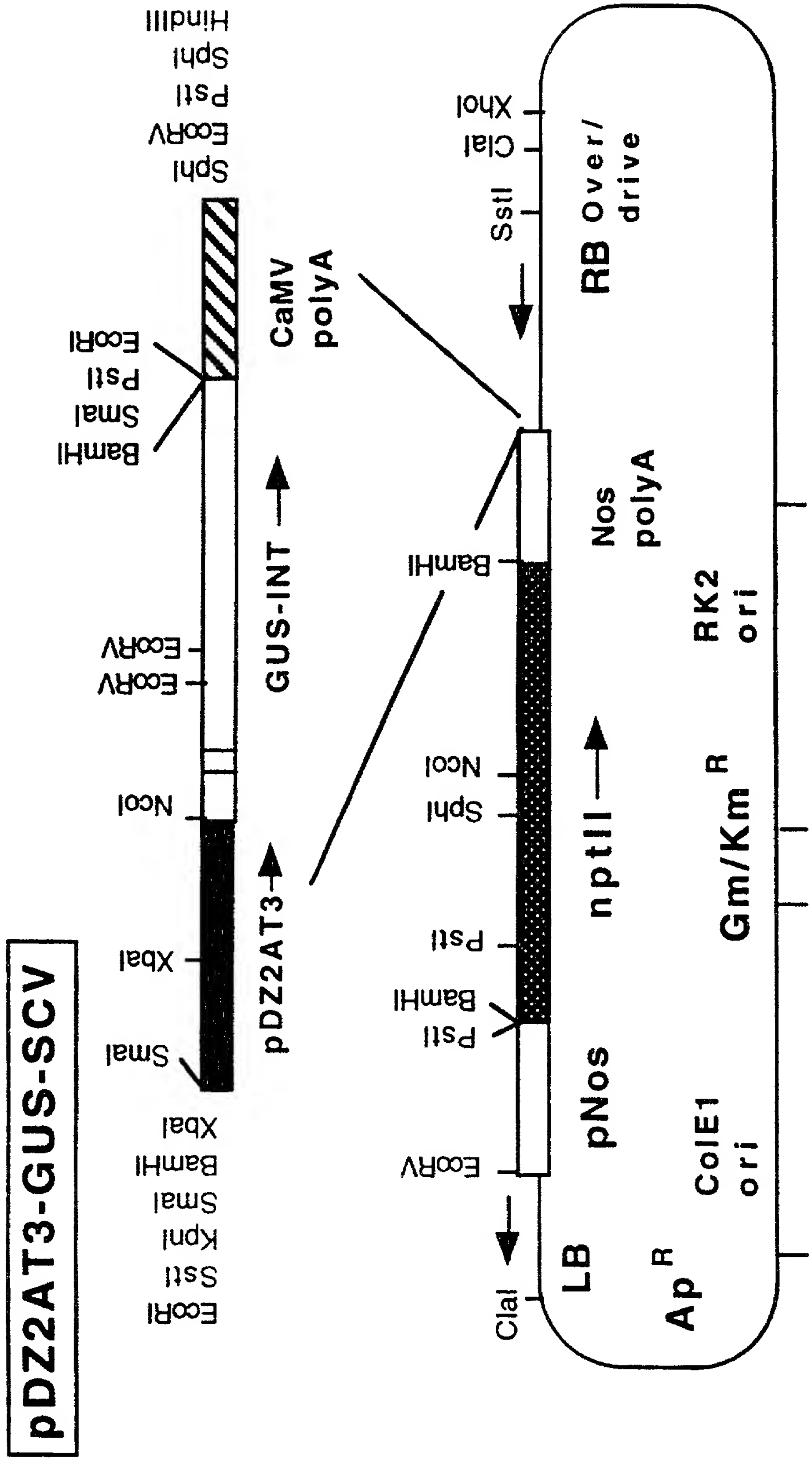

pDZ2AT3-GUS-SCV
HindIII
SphI
PstI
EcoRV
SphI
EcoRI
PstI
SmaI
BamHI
EcoRV
EcoRV
NcoI
XbaI
SmaI
XbaI
BamHI
SmaI
KpnI
SstI
EcoRI
CaMV polyA
GUS-INT
pDZ2AT3
XhoI
ClaI
SstI
BamHI
NcoI
SphI
PstI
BamHI
PstI
EcoRV
ClaI
RB Over/drive
Nos polyA
nptII
pNos
LB
RK2 ori
Gm/Km R
ColE1 ori
Ap R
SphI
KpnI
SstI
SphI

FIG.12

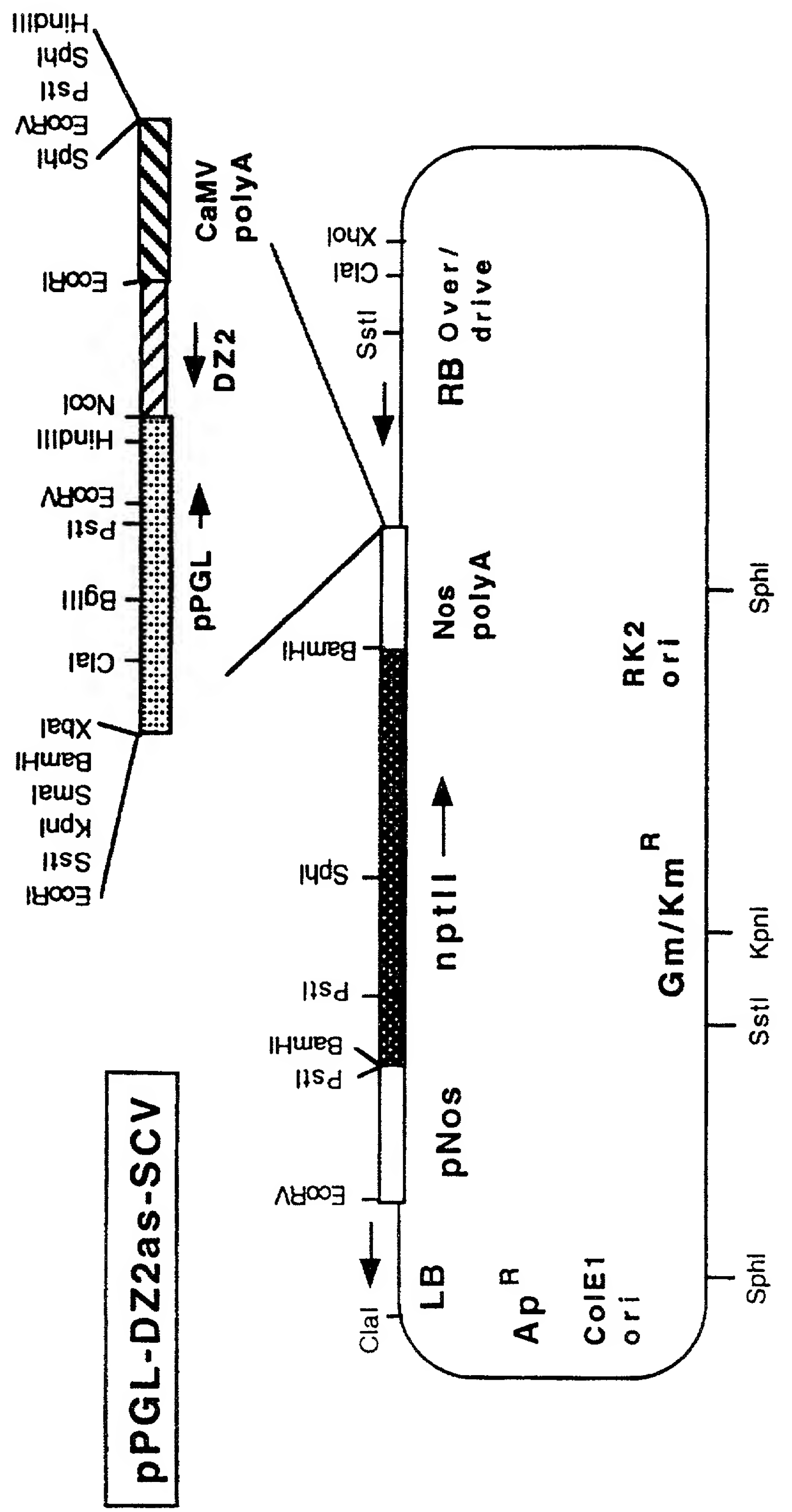

pPGL-DZ2as-SCV
HindIII
SphI
PstI
EcoRV
SphI
CaMV polyA
EcoRI
DZ2
NcoI
HindIII
EcoRV
PstI
pPGL
BglII
ClaI
XbaI
BamHI
SmaI
KpnI
SstI
EcoRI
XhoI
ClaI
SstI
RB
Over/drive
Nos polyA
BamHI
SphI
nptII
PstI
BamHI
PstI
pNos
EcoRV
ClaI
LB
Ap^R
ColE1 ori
RK2 ori
Gm/Km^R
SphI
KpnI
SstI
SphI

FIG. 13A

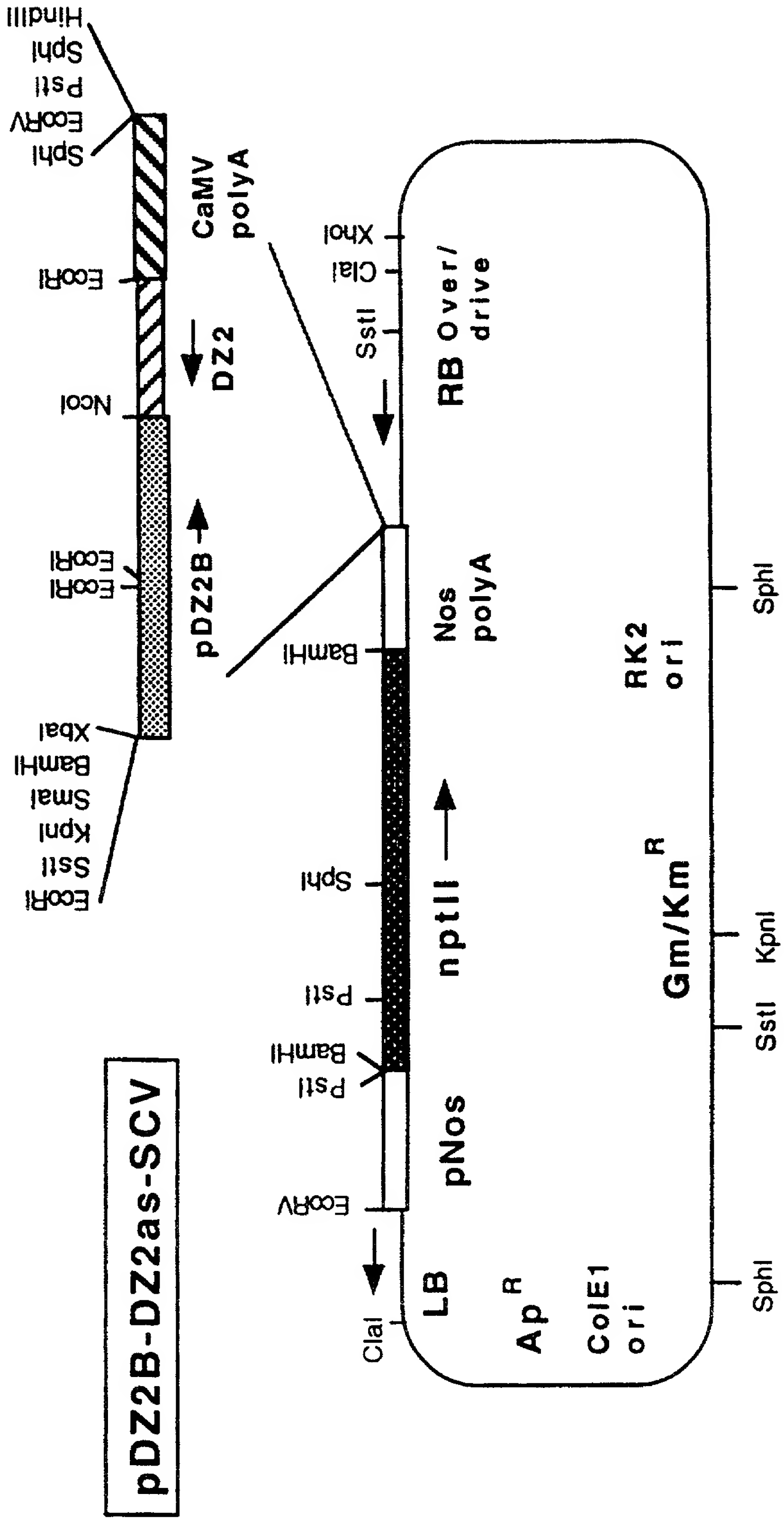

pDZ2B-DZ2as-SCV
HindIII
SphI
PstI
EcoRV
SphI
CaMV polyA
EcoRI
DZ2
NcoI
pDZ2B
EcoRI
EcoRI
XbaI
BamHI
SmaI
KpnI
SstI
EcoRI
XhoI
ClaI
SstI
RB Over/drive
Nos polyA
BamHI
SphI
nptII
PstI
BamHI
PstI
pNos
EcoRV
ClaI
LB
Ap R
ColE1 ori
RK2 ori
Gm/Km R
SphI
KpnI
SstI
SphI

FIG. 13B

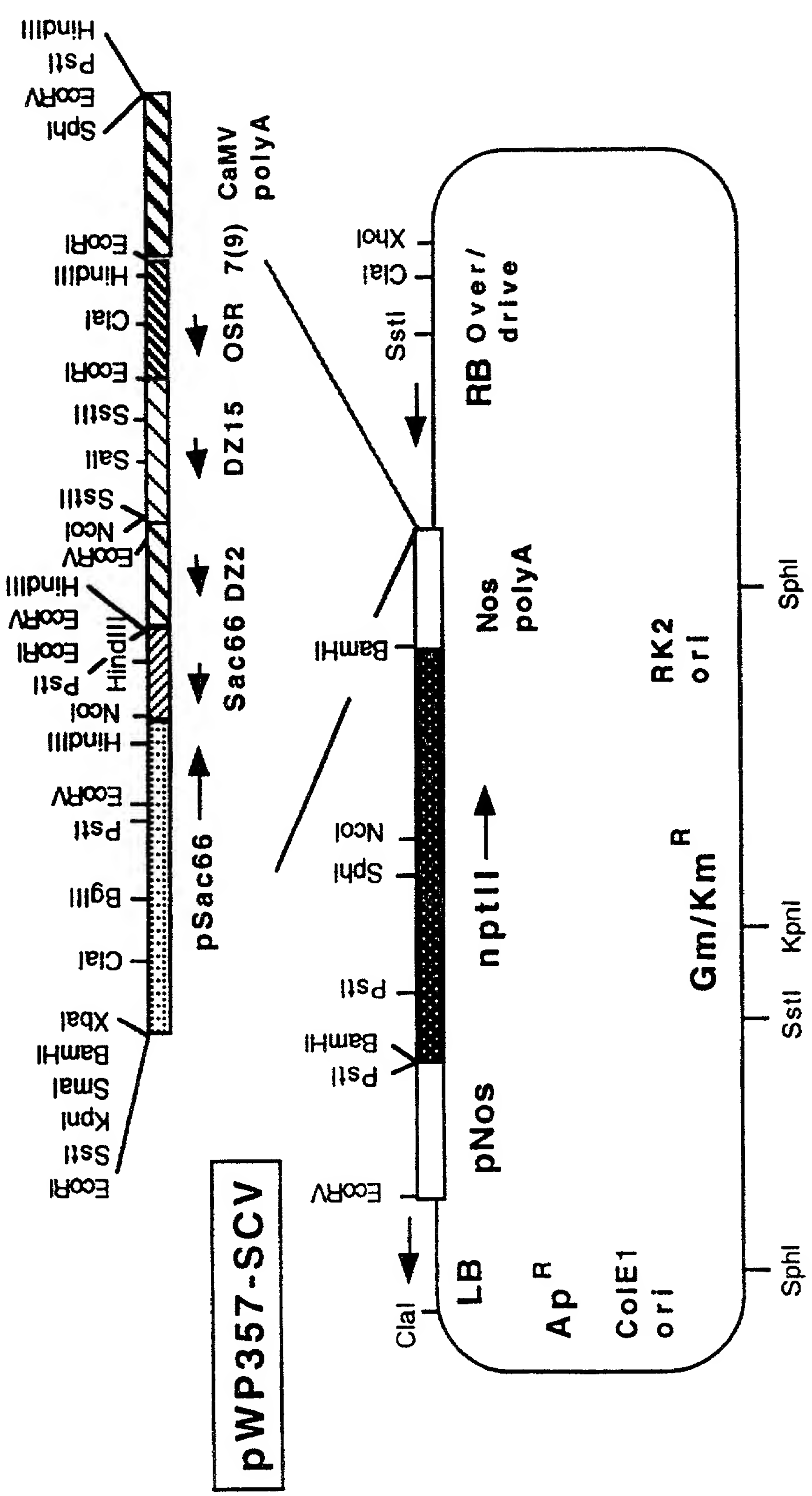

pWP357-SCV
HindIII
PstI
EcoRV
SphI
CaMV
polyA
EcoRI
HindIII
7(9)
ClaI
OSR
EcoRI
SstII
DZ15
SalI
SstII
NcoI
EcoRV
DZ2
HindIII
EcoRV
HindIII
EcoRI
Sac66
PstI
NcoI
HindIII
EcoRV
PstI
pSac66
BglII
ClaI
XbaI
BamHI
SmaI
KpnI
SstI
EcoRI
XhoI
ClaI
SstI
RB Over/
drive
Nos
polyA
BamHI
NcoI
SphI
nptII
PstI
BamHI
PstI
pNos
EcoRV
LB
ClaI
Ap^R
ColE1
ori
RK2
ori
Gm/Km^R
SphI
KpnI
SstI
SphI

FIG. 14

FIG. 15

```
GGCATCACGAGGGTACCCGTAAATCCCACCATACAACAAAGTTCTGTGAAAGTCTCCCAA 60

AAACTGCAAAGAGTCTCATATTAGTTCTTACTCTCAGAAATAAAACACAGTGTTTCTGAA 120

AAGATTAGCGTTTCAAACCCCGAAATGGCCCGTTGTCATGGAAGTCTTGCTATTTTCTTA 180
                         M  A  R  C  H  G  S  L  A  I  F  L  12

TGCGTTCTTTTGATGCTCGCTTGCTGCCAAGCTTTGAGTAGCAACGTAGATGATGGATAT 240
 C  V  L  L  M  L  A  C  C  Q  A  L  S  S  N  V  D  D  G  Y  32

GGTCATGAAGATGGAAGCTTCGAAACCGATAGTTTAATCAAGCTCAACAACGACGACGAC 300
 G  H  E  D  G  S  F  E  T  D  S  L  I  K  L  N  N  D  D  D  52

GTTCTTACCTTGAAAAGCTCCGATAGACCCACTACCGAATCATCAACTGTTAGTGTTTCG 360
 V  L  T  L  K  S  S  D  R  P  T  T  E  S  S  T  V  S  V  S  72

AACTTCGGAGCAAAAGGTGATGGAAAAACCGATGATACTCAGGCTTTCAAGAAAGCATGG 420
 N  F  G  A  K  G  D  G  K  T  D  D  T  Q  A  F  K  K  A  W  92

AAGAAGGCATGTTCAACAAATGGAGTGACTACTTTCTTGATTCCTAAAGGGAAGACTTAT 480
 K  K  A  C  S  T  N  G  V  T  T  F  L  I  P  K  G  K  T  Y  112

CTCCTTAAGTCTATTAGATTCAGAGGCCCATGCAAATCATTACGTAGCTTCCAGATCCTA 540
 L  L  K  S  I  R  F  R  G  P  C  K  S  L  R  S  F  Q  I  L  132

GGCACTTTATCAGCTTCTACAAAACGATCGGATTACAGTAATGACAAGAACCACTGGCTT 600
 G  T  L  S  A  S  T  K  R  S  D  Y  S  N  D  K  N  H  W  L  152
```

```
ATTTTGGAGGACGTTAATAATCTATCAATCGATGGCGGCTCGGCGGGATTGTTGATGGC 660
 I  L  E  D  V  N  N  L  S  I  D  G  G  S  A  G  I  V  D  G  172

AACGGAAAAATCTGGTGGCAAAACTCATGCAAAATCGACAAATCTAAGCCATGCACAAAA 720
 N  G  K  I  W  W  Q  N  S  C  K  I  D  K  S  K  P  C  T  K  192

GCGCCAACGGCTCTTACTCTCTACAACCTAAACAATTTGAATGTGAAGAATCTGAGAGTG 780
 A  P  T  A  L  T  L  Y  N  L  N  N  L  N  V  K  N  L  R  V  212

AGAAATGCACAGCAGATTCAGATTTCGATTGAGAAATGCAACAGTGTTGATGTTAAGAAT 840
 R  N  A  Q  Q  I  Q  I  S  I  E  K  C  N  S  V  D  V  K  N  232

GTTAAGATCACTGCTCCTGGCGATAGTCCCAACACGGATGGTATTCATATCGTTGCTACT 900
 V  K  I  T  A  P  G  D  S  P  N  T  D  G  I  H  I  V  A  T  252

AAAAACATTCGAATCTCCAATTCAGACATTGGGACAGGTGATGATTGCATATCCATTGAG 960
 K  N  I  R  I  S  N  S  D  I  G  T  G  D  D  C  I  S  I  E  272

GATGGATCGCAAAATGTTCAAATCAATGATTTAACTTGCGGCCCCGGTCATGGCATCAGC 1020
 D  G  S  Q  N  V  Q  I  N  D  L  T  C  G  P  G  H  G  I  S  292

ATTGGAAGCTTGGGGGATGACAATTCCAAAGCTTATGTATCGGGAATTAATGTGGATGGT 1080
 I  G  S  L  G  D  D  N  S  K  A  Y  V  S  G  I  N  V  D  G  312
```

FIG. 15 CONT'D

```
GCTACGCTCTCTGAGACTGACAATGGAGTAAGAATCAAGACTTACCAGGGAGGGTCAGGA 1140
 A  T  L  S  E  T  D  N  G  V  R  I  K  T  Y  Q  G  G  S  G  332

ACTGCTAAGAACATTAAATTCCAAAACATTCGTATGGATAATGTCAAGAATCCGATCATA 1200
 T  A  K  N  I  K  F  Q  N  I  R  M  D  N  V  K  N  P  I  I  352

ATCGACCAGAACTACTGCGACAAGGACAAATGCGAACAACAAGAATCTGCGGTTCAAGTG 1260
 I  D  Q  N  Y  C  D  K  D  K  C  E  Q  Q  E  S  A  V  Q  V  372

AACAATGTCGTCTATCGGAACATACAAGGTACGAGCGCAACGGATGTGGCGATAATGTTT 1320
 N  N  V  V  Y  R  N  I  Q  G  T  S  A  T  D  V  A  I  M  F  392

AATTGCAGTGTGAAATATCCATGCCAAGGTATTGTGCTTGAGAATGTGAACATCAAAGGA 1380
 N  C  S  V  K  Y  P  C  Q  G  I  V  L  E  N  V  N  I  K  G  412

GGAAAAGCTTCTTGCAAAAATGTCAATGTTAAGGATAAAGGCACCGTTTCTCCTAAATGC 1440
 G  K  A  S  C  K  N  V  N  V  K  D  K  G  T  V  S  P  K  C  432

CCTTAATTACTAAGTTGATTATGTAATATACATAAATACGTATTATATGTGGTTATAGAT 1500
 P                                                           433

GCCATCTATATCCTTATCTACGTATTGATTCTCGATATATATAGAAAACTAAGGATTTAT 1560

GGGAATATACATACAATAGTTGAGATAATTGTTGTCTTGTATATGGTTCACTGAAGTTGA 1620

TTGCTTGTCCACGAATAAATGAATAATGTCATTTGTC                        1657
```

FIG. 15 CONT'D

```
aggtgaccgttgctgatggcaatgtgctggtcaagcgagaggtagacggtggcttggaga
 V  T  V  A  D  G  N  V  L  V  K  R  E  V  D  G  G  L  E  T
cagttaaagtcaaattgccagctgtcattagcgccgacttgcggctcaatgagccgcggt
 V  K  V  K  L  P  A  V  I  S  A  D  L  R  L  N  E  P  R  Y
acgctactctgcccaatatcatgaaggccaagaagaagcccatcaaaaagctcacagcca
 A  T  L  P  N  I  M  K  A  K  K  K  P  I  K  K  L  T  A  T
cagatgtcggtgtggacttggcgccacgtcaacaagtgttgagcgtagaagacccgccca
 D  V  G  V  D  L  A  P  R  Q  Q  V  L  S  V  E  D  P  P  T
ccagacaggctggttccattgtgcctgatgtcgacactctcatcaccaagttgaaagaaa
 R  Q  A  G  S  I  V  P  D  V  D  T  L  I  T  K  L  K  E  K
agggtcatttgtaatgcaatgtcaccaatacagttgttttagttcttacaaattcttcgt
 G  H  L  *
gaggttttcagctgttaccaataatatttttcaaaatcgattttattttacttgtaatt

taaaagatcaaatattaatacaatgaacattttgtaacagcaatctttttttatattt

tggagatttcatcgacttatgtcataattatttttatcaatttattgttgtttgttagtg

atataataaagtatgttttctggtcaaaa
```

FIG. 16

OSR79 306bp

```
5'  ggttgggtcgaaccataggtggaaagcttctttctctctcgcttgacaaatcctctggtt
     L  G  R  T  I  G  G  K  L  L  S  L  S  L  D  K  S  S  G  S

    cgggttttcagtcccatcaggagtttctctatggtaaagctgaggttcaaatgaaacttg
     G  F  O  S  H  Q  E  F  L  Y  G  K  A  E  V  O  M  K  L  V

    tccctggtaactctgctggaacagtcacaacattctatcttaaatcaccgggaactacat
     P  G  N  S  A  G  T  V  T  T  F  Y  L  K  S  P  G  T  T  W

    gggatgagatcgatttcgagttcttgggaaacataagtggccatccctatactctccata
     D  E  I  D  F  E  F  L  G  N  I  S  G  H  P  Y  T  L  H  T

    ctaatgtttacacacgaaggctctggagacaaagaacagcagtttcatctatggttcgac
     N  V  Y  T  R  R  L  W  R  Q  R  T  A  V  S  S  M  V  R  P

    ccgacc     3'
     D
```

FIG. 17

Appl. No. 09/646,679
(U.S. Nat'l Phase of PCT/GB99/00905)
Docket Number: 0623.0890000
K&S Ref: KVC/PS/CF/P20895US

POWER OF ATTORNEY FROM ASSIGNEE

Biogemma UK Limited, a corporation of Great Britain, having a principal place of business at 200 Science Park, Milton Road, Cambridge CB4 0GZ, Great Britain, is assignee of the entire right, title and interest for the United States of America (as defined in 35 U.S.C. § 100), by reason of an Assignment to the Assignee executed on ✓ 7 December 2000, 8 December 2000 and 11 December 2000 of an invention known as Signal Transduction Protein Involved In Plant Dehiscence (Attorney Docket No. 0623.0890000), which is disclosed and claimed in a patent application of the same title by the inventor(s) Wyatt Paul, Jeremy Alan Roberts, and Catherine Whitelaw (said application filed on September 20, 2000 at the U.S. Patent and Trademark Office, having Application Number 09/646,679, which is the U.S. National Phase of PCT/GB99/00905; international filing date: 22 March 1999).

The Assignee hereby appoints the following U.S. attorneys to prosecute this application and any continuation, divisional, continuation-in-part, or reissue application thereof, and to transact all business in the U.S. Patent and Trademark Office connected therewith: Robert Greene Sterne, Esq., Reg. No. 28,912; Edward J. Kessler, Esq., Reg. No. 25,688; Jorge A. Goldstein, Esq., Reg. No. 29,021; Samuel L. Fox, Esq., Reg. No. 30,353; David K.S. Cornwell, Esq., Reg. No. 31,944; Robert W. Esmond, Esq., Reg. No. 32,893; Tracy-Gene G. Durkin, Esq., Reg. No. 32,831; Michele A. Cimbala, Esq., Reg. No. 33,851; Michael B. Ray, Esq., Reg. No. 33,997; Robert E. Sokohl, Esq., Reg. No. 36,013; Eric K. Steffe, Esq., Reg. No. 36,688, Michael Q. Lee, Esq., Reg. No. 35,239; Steven R. Ludwig, Esq., Reg. No. 36,203; Raz E. Fleshner, Esq., Reg. No. 34,331; John M. Covert, Esq., Reg. No. 38,759; and Linda E. Alcorn, Esq., Reg. No. 39,588. The Assignee hereby grants said attorneys the power to insert on this Power of Attorney any further identification that may be necessary or desirable in order to comply with the rules of the U.S. Patent and Trademark Office.

Send correspondence to:

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1100 New York Avenue, N.W.
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Washington, D.C. 20005-3934
U.S.A.

Direct phone calls to 202-371-2600.

FOR: Biogemma UK Limited
SIGNATURE: ✓
BY: ✓ Tina Lorraine Barsby
TITLE: ✓ Company Secretary
DATE: ✓ 18 December 2000

RECEIVED 13 DEC 2000

Declaration for Patent Application

Appl. No. 09/646,679
(U.S. Nat'l Phase of PCT/GB99/00905)
Docket Number: 0623.0890000
K&S Ref: KVC/PS/CF/P20895US

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled Signal Transduction Protein Involved in Plant Dehiscence, the specification of which is attached hereto unless the following box is checked:

☒ was filed on March 22, 1999 (International Filing Date);
as United States Application Number or PCT International Application Number PCT/GB99/00905 (U.S. Appl. No. 09/646,679); and
was amended on ____________ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s) Claimed			Priority	
9806113.8	Great Britain	20 March 1998	☒ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		
			☐ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application No.)	(Filing Date)
(Application No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

PCT/GB99/00905	March 22, 1999	Abandoned
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)

Appl. No. 09/646,679
(U.S. Nat'l Phase of PCT/GB99/00905)
Docket Number: 0623.0890000
K&S Ref: KVC/PS/CF/P20895US

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Washington, D.C. 20005-3934

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(202) 371-2600

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Wyatt Paul	
Signature of sole or first inventor ✓ ✓	Date
Residence Cambridge, Great Britain	
Citizenship Great Britain	
Post Office Address Biogemma UK Limited, 200 Science Park, Milton Road, Cambridge, CB4 OGZ, Great Britain	
Full name of second inventor Jeremy Alan Roberts	
Signature of second inventor J. A. Roberts ✓ ✓ 8/12/00.	Date
Residence Leicestershire, Great Britain	
Citizenship Great Britain	
Post Office Address University of Nottingham, Division of Plant Science, School of ~~Biological Sciences~~ Biosciences R., Sutton Bonington Campus, Loughborough, Leicestershire ~~LE~~12 5RD, Great Britain GBN	
Full name of third inventor Catherine Whitelaw	
Signature of third inventor ✓ ✓	Date
Residence Beltsville, Maryland	
Citizenship Great Britain	
Post Office Address USDA/ARS/SARL, Room 208, Building 006 BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA	

P:\USERS\PChoi\0623\089\declaration wpd

(Supply similar information and signature for subsequent joint inventors, if any)

Declaration for Patent Application

Appl. No. 09/646,679
(U.S. Nat'l Phase of PCT/GB99/00905)
Docket Number: 0623.0890000
K&S Ref: KVC/PS/CF/P20895US

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled Signal Transduction Protein Involved in Plant Dehiscence, the specification of which is attached hereto unless the following box is checked:

☒ was filed on March 22, 1999 (International Filing Date);
as United States Application Number or PCT International Application Number PCT/GB99/00905 (U.S. Appl. No. 09/646,679); and
was amended on ________________(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s) Claimed			Priority	
9806113.8	Great Britain	20 March 1998	☒ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		
			☐ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application No.)	(Filing Date)
(Application No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

PCT/GB99/00905	March 22, 1999	Abandoned
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)

Send Correspondence to:

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934

Direct Telephone Calls to:

(202) 371-2600

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Wyatt Paul	
Signature of sole or first inventor ✓ ✓	Date
Residence Cambridge, Great Britain	
Citizenship Great Britain	
Post Office Address Biogemma UK Limited, 200 Science Park, Milton Road, Cambridge, CB4 OGZ, Great Britain	
Full name of second inventor Jeremy Alan Roberts	
Signature of second inventor ✓ ✓	Date
Residence Leicestershire, Great Britain	
Citizenship Great Britain	
Post Office Address University of Nottingham, Division of Plant Science, School of Biological Sciences, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, Great Britain	
Full name of third inventor Catherine Whitelaw	
Signature of third inventor ✓ C Whitelaw ✓ 11/12/00	Date
Residence Beltsville, Maryland	
Citizenship Great Britain	
Post Office Address USDA/ARS/SARL, Room 208, Building 006 BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA MW	

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(Supply similar information and signature for subsequent joint inventors, if any)

Declaration for Patent Application

Appl. No. 09/646,679
(U.S. Nat'l Phase of PCT/GB99/00905)
Docket Number: 0623.0890000
K&S Ref: KVC/PS/CF/P20895US

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled Signal Transduction Protein Involved in Plant Dehiscence, the specification of which is attached hereto unless the following box is checked:

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1-00

Full name of sole or first inventor Wyatt Paul	
Signature of sole or first inventor ✓ W. Paul	Date ✓ 7/12/00
Residence Cambridge, Great Britain	
Citizenship Great Britain	
Post Office Address Biogemma UK Limited, 200 Science Park, Milton Road, Cambridge, CB4 OGZ, Great Britain GBN	
Full name of second inventor Jeremy Alan Roberts	
Signature of second inventor ✓	Date ✓
Residence Leicestershire, Great Britain	
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Full name of third inventor Catherine Whitelaw	
Signature of third inventor ✓	Date ✓
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(Supply similar information and signature for subsequent joint inventors, if any)